THE

AMERICAN JOURNAL

OF THE MEDICAL SCIENCES

MAY, 1939

ORIGINAL ARTICLES.

STUDIES IN DYSTROPHIA MYOTONICA. I. HEREDITARY ASPECTS.

By Abe Ravin, M.D.,
INSTRUCTOR IN MEDICINE, UNIVERSITY OF COLORADO SCHOOL OF MEDICINE,

AND

James J. Waring, M.D.,
PROFESSOR OF MEDICINE, UNIVERSITY OF COLORADO SCHOOL OF MEDICINE, DENVER, COLORADO.

(From the Department of Medicine, University of Colorado School of Medicine and Hospitals.)

Dystrophia myotonica is of interest to workers in many fields. The neurologist, the physiologist, the ophthalmologist, the endocrinologist, the geneticist, the internist, all have something to learn from this fascinating disease. The neurologist must differentiate the muscular atrophy of this disease from other muscular dystrophies and atrophies. Although the highly characteristic pattern of the atrophy is important, that is, the almost constant involvement of the muscles of the forearm, the muscles of the face, the sterno-mastoids, and the dorsi-flexors of the feet, the differentiation is usually made easy by the presence of the other features of the disease. The physiologist can learn much about normal and abnormal muscle behavior from the study of the myotonia which occurs in this disease. This disturbance of muscle function is characterized by a persistence of the state of contraction of a muscle after cessation of the stimuli producing the contraction; thus, if a patient grasps an object with his hand, he may not be able to release it for a number of seconds because of a persistence of the contraction of the flexors of the fingers. In this disease, myotonia is seen most commonly in the muscles of the forearm and hand, but it may be more widespread; it is rarely as generalized as in myotonia congenita (Thom-

sen's disease). The ophthalmologist is interested in the almost constant occurrence of a very characteristic type of presenile cataract. The endocrinologist ponders the apparent polyglandular involvement, so strikingly evident in most of these patients. The basal metabolic rate is usually low and the thyroid is frequently enlarged, but the blood cholesterol is normal and myxedematous deposits are lacking. Cataract and the frequent presence of a positive Chvostek suggest hypoparathyroidism, but the blood calcium and phosphorus are usually normal. Testicular atrophy and baldness are common. Geneticists have studied this disease in an effort to express the method of inheritance in classical mendelian terms. A disease of such varied manifestations must belong in final analysis to the internist who interprets each of the many signs as evidence of the presence of a "more widespread morbid process."

Study of the inheritance of the muscular dystrophies serves as a valuable aid in the nosological distinction of these diseases. Clinical distinction between two very similar diseases may be confirmed by the demonstration of different types of inheritance. This fact has been stressed by Barker[1] who cites as an example the case of the similar but clinically differentiated Erb-Duchenne and Landouzy-Dejerine types of muscular dystrophy. In a muscular dystrophy, probably of the Erb-Duchenne type, studied by Minkowski and Sidler,[17] the inheritance is of double mendelian recessive nature. The Landouzy-Dejerine type of muscular dystrophy, however, exhibits mendelian dominance. A similar observation can be made in the case of dystrophia myotonica and myotonia congenita. These diseases, confused for many years because of the occurrence in both of myotonia, have been separated on the basis of clinical findings. Again we find that differences in the methods of inheritance justify the separation of these diseases. Myotonia congenita, as the studies of Nissen[18] show, is transmitted as a pure mendelian dominant. Nissen, tracing the disease in his own family from 1740, was able to show that the disease was inherited without interruption from one generation to another; myotonic descendants were always from myotonic parents and in no case did one find an individual without myotonia whose descendants showed myotonia. On the other hand, dystrophia myotonica occurs commonly in the children of apparently normal parents, and is infrequently seen in two successive generations. Dystrophia myotonica exhibits what appears to be a very irregular type of inheritance. The nature of this inheritance is the object of this report.*

* We are familiar with the work of Boeters[4] in which the author presents a number of pedigrees to prove the occurrence of both dystrophia myotonica and myotonia congenita in the same families and concludes that the two diseases are different external manifestations of the same inherited defect. We feel, however, that he has failed to differentiate clearly the two diseases. He divides the cases into those with myotonia alone, which he calls myotonia congenita, and those with myotonia associated with dystrophic and endocrine anomalies, among which he places dystrophia

Description of Families. B. FAMILY (Figs. 1 and 2). *Generation* I.—All born in America. *2** believed to have been of Scotch ancestry, *3* of "Pennsylvania Dutch," and *4* of Scotch-Irish.

Generation II.—Nothing remarkable.

Generation III.—*1.* M. W. B., aged 62; weakness and atrophy of muscles of forearm and hand for over 14 years; myotonia in handgrasp for 4 or 5 years; difficulty in walking for over 10 years; intolerance to cold; baldness; typical "myopathic" facies; marked atrophy of the sterno-cleido-mastoid muscles and the muscles of the forearms and hands; mechanical myotonia in the tongue and hand muscles.

2. J. B., aged 60; weakness of the handgrasps for at least 13 years; difficulty in walking for several years; generalized weakness and loss of weight in recent years; baldness; typical "myopathic" facies; testicular atrophy; early cataract bilaterally; generalized muscular atrophy; voluntary myotonia in handgrasps; myotonic reaction in muscles of forearm and hand.

Generation IV.—*3.* R. B., aged 27; weakness in handgrasps first noticed 10 years ago; some difficulty in walking noticed in last year; intolerance to cold; loss of facial expression; early cataract bilaterally; testicular atrophy; marked atrophy of sterno-cleido-mastoids; voluntary myotonia in handgrasps; myotonic reaction in muscles of forearm and hands.

4. M. J. B., aged 20; voluntary myotonia in handgrasps since the age of 13; some weakness and atrophy of the sterno-cleido-mastoid muscles; early cataract present bilaterally; mechanical myotonia in tongue muscles and muscles of forearms and hands.

M. FAMILY (Fig. 3). *Generation* I.—*1* and *2* are believed to have been born in America. *4* and *5* came from Germany.

Generation II.—*1* did not talk plainly. *5* had a cataract operation at 65 years. *6* began to have cataracts at about 50 years and was operated upon for them at about 60 years. *12* was an invalid for the last 35 years of her life and was unable to leave her wheel chair for many years before her death at 64 years. An obituary note states that she had "locomotor ataxia."

Generation III.—*18.* N. M., aged 59; myotonia in handgrasps for 7 years; difficulty in walking for 1½ years; cataracts for at least 13 years; marked atrophy of the sterno-cleido-mastoid muscles; myotonic reaction in muscles of the forearm and hand.

---

myotonica. This seems a false division for the following reasons: (1) The presence of myotonia alone does not mean that the case may not be one of dystrophia myotonica, for it is well recognized that early in the course of this disease the myotonia may be the only symptom. (2) Myotonia with dystrophic signs of irregular pattern and type does not constitute the syndrome of dystrophia myotonica. Dystrophia myotonica presents a clinical picture almost monotonous in its uniformity.

This false division may completely distort the interpretation of a pedigree as will be seen by examination of the pedigree of Boeters' Case 1. In this family are found 10 cases of myotonia, 7 of which Boeters classifies as myotonia congenita and 3 of which he classifies as myotonia with dystrophic and endocrine anomalies. We see no reason for this division and think all the cases in this family are myotonia congenita because: (1) In the 10 cases there is no single case of muscular atrophy or of cataract. (2) In our experience the presence of atrophy and cataract in dystrophia myotonica has been more constant than the presence of myotonia. (3) The ages of onset are stated to be about 20 years in 1 case, in "youth" in 3 cases, and in the other cases 13, 12, 11, before 10, 4 and 3 years of age: the earliest age of onset in the pedigree, 3 years, occurred in the mother of 8 of the cases. This onset of the disease in youth in practically every case is the expected finding in a pedigree of myotonia congenita and varies markedly from the experience of workers on dystrophia myotonica as seen in Table 1.

*The numbers in italics refer to the members of the generation indicated. In the figures these members are numbered consecutively from left to right.

19. J. L., aged 56; myotonia in handgrasps for the last 8 years; some difficulty in walking up steps during the last few months; incipient cataracts found 3 years ago; mechanical myotonia in muscles of tongue, forearm and hand.

20. C. M., aged 53; clamping of hands on tools; no other symptoms or findings.

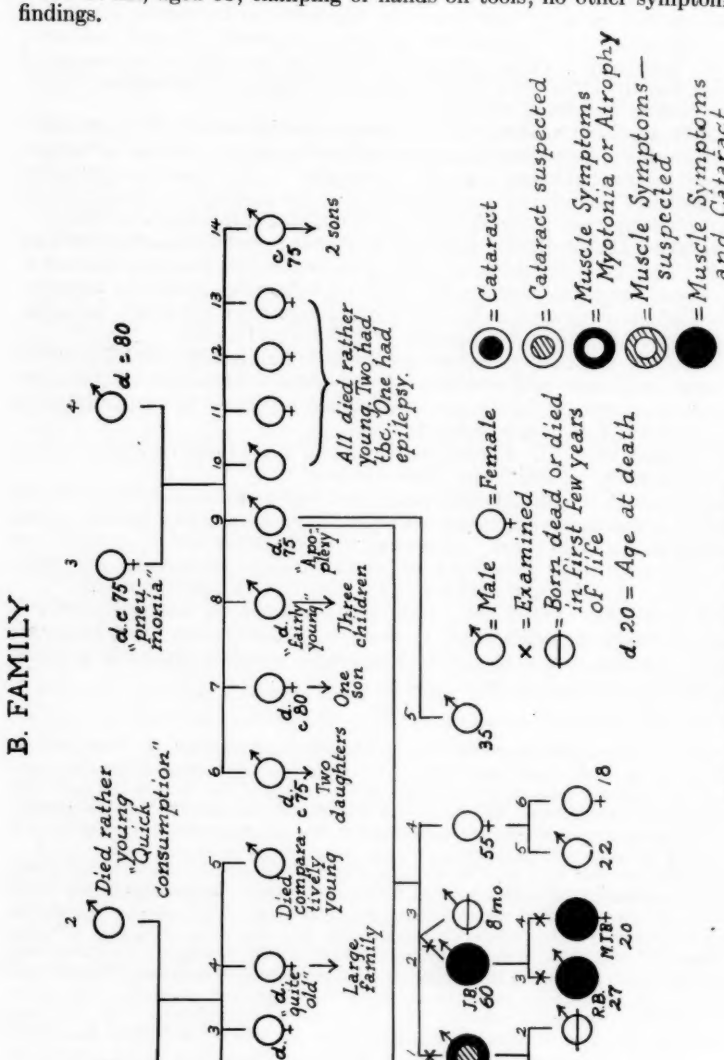

FIG. 1.—Pedigree of the B. Family.

25. O. M., aged 43; some weakness in his hands since age of 27; told he had cataracts at 30; noticed myotonia in handgrasps at 33 and in legs during last 4 or 5 years; intolerant to cold; "hatchet" facies; "steppage" gait; testicles atrophic; myotonic reaction in muscles of forearm and hand.

Generation IV.—*17.* H. M., aged 22, has cataracts of the type described as typical for dystrophia myotonica.

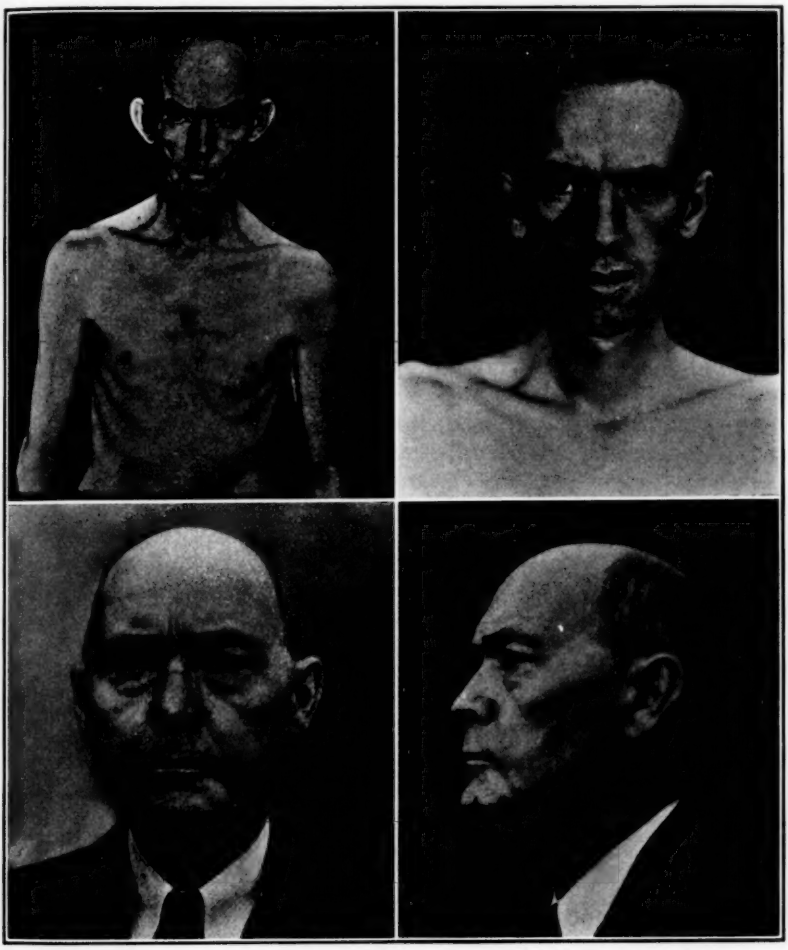

FIG. 2.—Some members of the B. Family. Upper left, J. B. (III *2* in Fig. 1). Upper right, R. B. (IV *3*), son of J. B. Lower right and left, M. W. B. (III *1*), brother of J. B.

PF. FAMILY (Fig. 4). *Generation* I.—*1* and *3* were born in America. *4* and *5* were born in Germany.

Generation II.—*6* was operated upon for cataract at about 55 years of age. He died of diabetes but had diabetes only a few years before his death.

Generation III.—*2.* N. Pf., aged 54; myotonia in handgrasps for 15 years; weakness of handgrasps, especially in last 9 years; "slapping" gait

for about 5 years; cataract operation 2 years ago; baldness; marked atrophy of muscles of forearm and of sterno-cleido-mastoid muscles; myotonic reaction in muscles of forearm and hand; "myopathic" facies.

3. R. Pf., died at about 33 years of "heart attack"; cataract operation at 30 years; trouble with hands and legs for several years before his death.

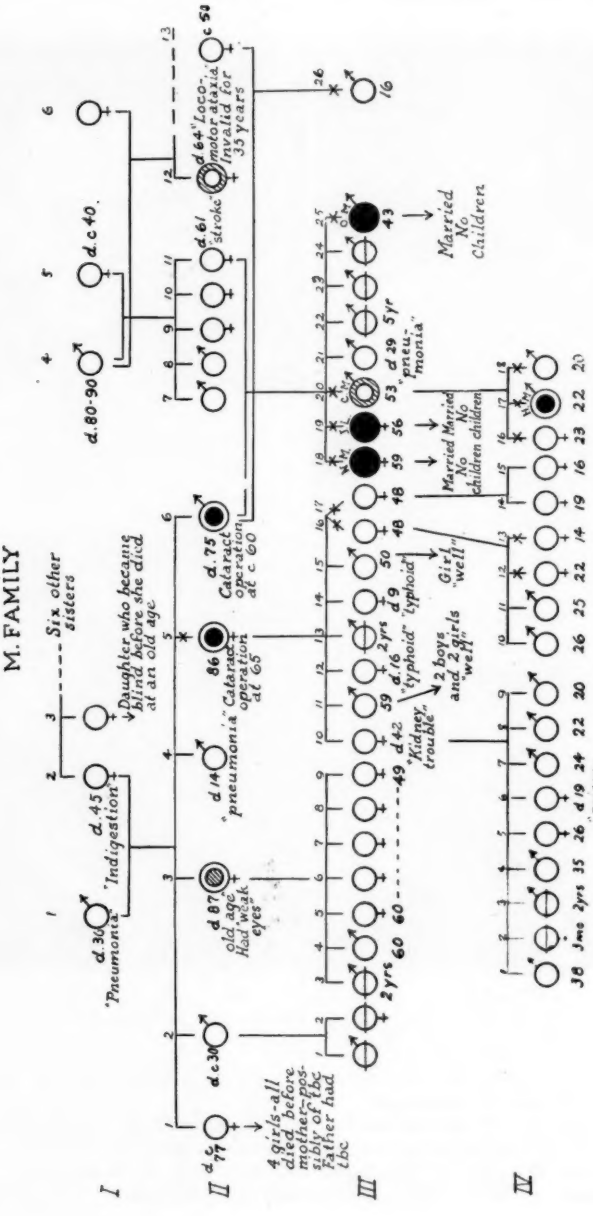

FIG. 3.—Pedigree of the M. Family. (Legend as in Fig. 1.)

4. M. S., died at 42 years following an operation for an abdominal tumor; cataract operation at 37 years; trouble with hands for several years before death.

5. R. R., aged 48; marked difficulty with hands for a number of years; cannot walk very well; difficulty in talking; poor vision for years.

D. FAMILY (Fig. 5). Except for some members in *Generation* IV, all members of this family were born in Sweden.

Generation I.—Nothing remarkable.

Generation II.—*14* had cataract operation at the age of 61 and again at 64. *15* died at 50 years following a cataract operation. She is said to have been deaf and dumb as a result of a childhood disease.

Generation III.—*2.* J. M., aged 46; myotonia present for 23 years; weakness of hands moderate in last 13 years; difficulty in walking for last 3 years; cataract operations 8 and 5 years ago; "myopathic" facies; marked atrophy of sterno-cleido-mastoid muscles; myotonic reaction in muscles of forearms and hands.

8. S. E., aged 34; has no complaints but has typical early cataracts.

9. Mrs. J., aged 31; has been operated upon for cataracts in the last year.

Discussion. The classical investigations of Fleischer[6a] in 1918 confirmed and emphasized the hereditary nature of dystrophia myotonica. The details of the method of inheritance have been determined slowly and can be expressed today in terms familiar to the experimental geneticist.

The fact that the parents of most patients with dystrophia myotonica are apparently normal suggests a recessive inheritance. The following observations, however, indicate that the factor responsible for the disease acts as a mendelian dominant:* (1) if the disease were recessive, the number of consanguineous marriages among the parents of the affected patients should be greatly increased since, in this case, the rarer the character in the general population, the more often will it be found that persons exhibiting the character are the offspring of the marriages of near kin.[2] Consanguineous marriages, however, are infrequent among the parents of patients with dystrophia myotonica. Not one occurred in any of our families. In his remarkable investigation of an affected family through six generations, Fleischer[6b] found no evidence that consanguineous marriages played any part in the manifestation of the disease. (2) Once the disease has become manifest in a family, affected individuals transmit it to their children. All instances of such trans-

* The two contrasting characters that are produced by the two differing members of a pair of chromosomes are spoken of as allelomorphs. The dominant character is that member of the pair of allelomorphs which, when both contrasting characters are present, predominates over the other, the recessive character, in its manifestations. The recessive character will, therefore, not be manifest unless present by itself, that is, in both members of the chromosome pair. When a character is present in both members of the chromsome pair, the individual is homozygous for that character. If the character is present in only one of the chromosomes, the individual is heterozygous for that character. In speaking of "dystrophia myotonica" as a dominant character, the contrasting or allelomorphic character is, of course, "normal." Since the chromosome pairs of the child are made up of one from each of the parents, a recessive character will become manifest only if each of the parents transmits it to the child. A dominant character, however, will be manifest in the child if only one parent transmits it.

mission which could be found in the literature have been tabulated in Table 1. Direct transmission of a disease from parent to child is strongly suggestive of dominant heredity, because, in the case of a dominant character, only one parent in each generation need transmit the defective gene; whereas, in the case of a recessive character, both parents in two successive generations must transmit the defective gene. If the disease is rare, the chance meeting in two succes-

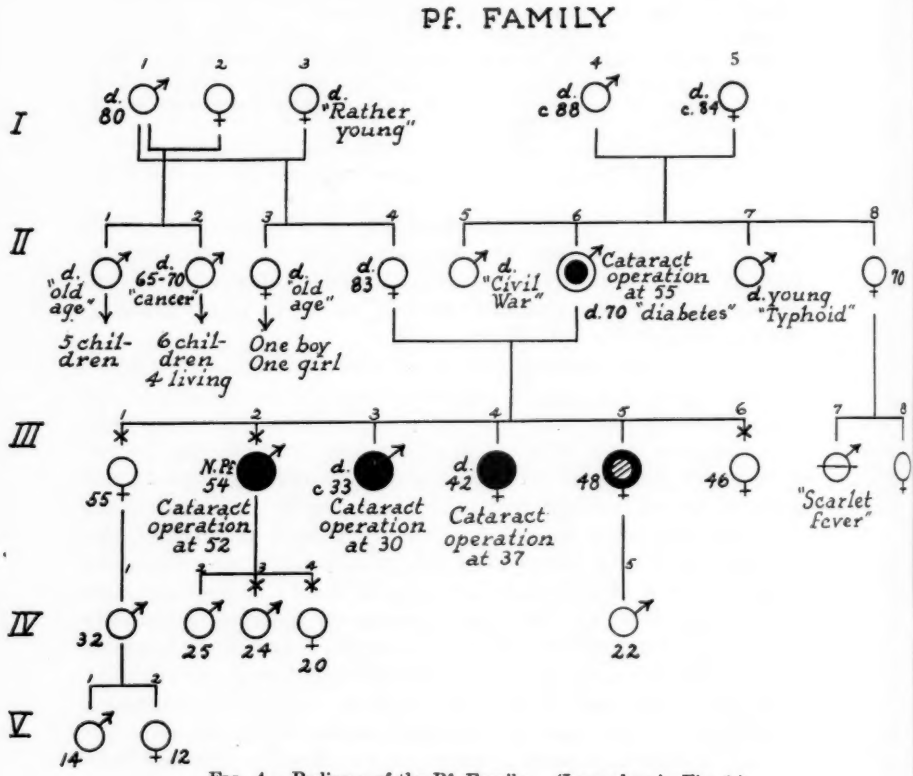

FIG. 4.—Pedigree of the Pf. Family. (Legend as in Fig. 1.)

sive generations of carriers of the disease gene is very unlikely. In the B. family (Fig. 1), if the gene is recessive, not only J. B. and his parents must be considered as carriers of the gene, but also the wife of J. B. (3) Maas,[15] Boeters[4] and Kolb *et al.*[13] report the occurrence of the disease in half brothers and sisters. A recessive character would require the participation of three carriers and, therefore, would be very unlikely. In the case of a dominant character, only the common parent need be affected. (4) Henke and Seeger's[12] investigation of their own material and the sibships reported in the

literature indicated that the percentage of affected individuals in the sibships was very close to 50. This was true in sibships of an affected parent as well as in sibships of apparently normal parents. This is the expected percentage in the case of a single factor dominant inheritance where the parent is heterozygously affected. In a

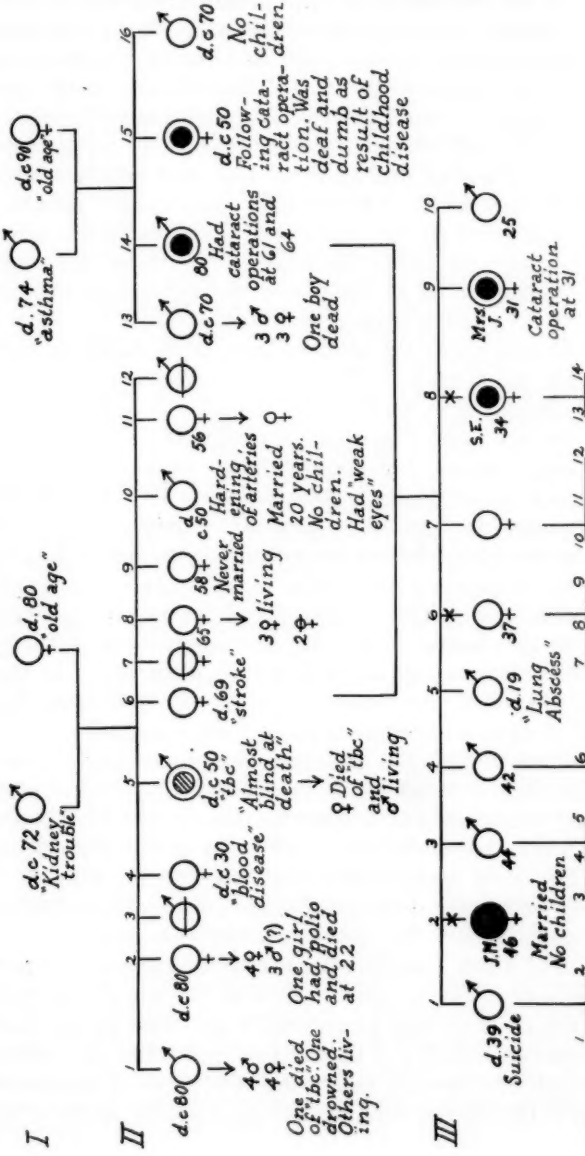

FIG. 5.—Pedigree of the D. Family. (Legend as in Fig. 1.)

rare disease the number of homozygously affected individuals would be too small to be significant. With a single factor recessive inheritance, only 25% of the siblings should be affected if the parents are apparently normal but heterozygous. Where one of the parents is affected the percentage of affected children would be 50 if the other parent is a carrier and zero if the other parent is normal. If a group of affected parents are considered, however, the percentage of affected children would be very small because the number of marriages of affected parents to normal individuals would greatly exceed the number of marriages to carriers of a rare disease. The percentage of affected individuals in the sibships in our pedigrees is over 50 and is probably explained by the error inherent in small numbers and the selection of material which results because the greater the number of affected individuals in a sibship the more apt the sibship is to come under observation. If those cases labeled suspicious are considered as unaffected, the percentage of affected individuals in the families studied by Maas[15] is 60.

These observations would seem to leave little doubt concerning the dominant inheritance. That this dominance is not typical, however, is obvious from the fact that the parents and grandparents of most of the patients are normal. A dominant factor may apparently skip a generation; but the occurrence of unaffected parents is too general to be explained in this manner. We have, therefore, a strong argument for a modified dominant inheritance. Investigation of the causes for the infrequency of the occurrence of dystrophia myotonica in two or more generations furnishes a clue to the nature of the modification. The causes probably are as follows: (1) Patients with the disease show a marked infertility and, therefore, have few descendants. Of 10 individuals, including 3 in the Pf. family not examined but unquestionably affected, who showed evidence of moderate or severe muscular signs and who were married, 5 have had no children and the remaining 5 have a total of 8 children (distributed as follows: 3, 2, 1, 1, 1). Since only 4 of these children may be expected to develop the disease and the 4 cases can occur in as few as 2 or 3 sibships, it is evident that of the original 10 cases only 2 to 4 are likely to have children with the disease. This number may be further decreased by the failure of the children to reach an age necessary for the disease to become manifest. (2) Examination of Table 1 reveals the very interesting and important fact that in every case, without exception, the age of onset of the disease in the children was at an earlier age than in the parents. This phenomenon, which will be discussed later, has been observed in other hereditary diseases and called the "law of anticipation." If the onset of the disease in an individual occurs at an age at which

he has already had children, his children and certainly his grandchildren will develop the disease at an age which will prevent further propagation. This becomes even more likely because of the frequently observed increasing severity of the disease in each succeeding generation.*

These observations in regard to the earlier age of onset and increasing severity of the disease in succeeding generations suggest an explanation for the occurrence of the disease in the children of apparently normal parents. It appears possible that the parent who carries the anlage for the disease either may have the disease in a very mild form or may not live long enough for it to develop. The first individual in a family who shows the complete syndrome usually manifests it in the third or fourth decade, and by the time medical advice is sought the parents usually are either dead or for other reason are not available for examination. Study of pedigrees reveals in the ancestors, however, the frequent occurrence of cataract, one of the most characteristic signs of dystrophia myotonia. Fleischer[6a] has offered evidence that the occurrence of this cataract also presents the remarkable phenomenon of anticipation; that is, the age of onset is earlier in each succeeding generation. In the early generations occur senile cataracts, in later generations presenile cataracts, when the complete syndrome appears the cataracts may occur in youth. In the pedigree here reported it will be noted that one of the parents of each of 3 affected sibships was operated upon for cataract (at ages of 55, 60 and 61) and 2 aunts were operated upon for cataract (at ages of 50 and 65). The parents of dystrophic patients appear, therefore, to manifest the inherited defect but in a limited form. Careful investigation of these individuals possibly would show that they have other mild evidences of the disease, as suspected by Curschmann[5b] and proved by several case histories in Maas' article.[15]

We may summarize this discussion as follows: The disease appears to be due to a dominant mutation, which is at first manifest by no signs or by very slight and few signs, notably cataract. The gene may be transmitted through several generations with no apparent manifestations, as shown by pedigrees in which apparently scattered cases of dystrophia myotonica may be traced back six or seven generations to a common ancestor. In those families where cataract occurs, its onset at an earlier age in succeeding generations is evidence of the presence of the defective gene. Finally, in one generation the disease becomes severe enough and sets in early enough to be clearly recognized. Individuals with the disease now transmit it to their children as a simple dominant. The onset of the

* In comparing severity of a disease it must be remembered that the duration of the disease is important and that the comparative severity of the disease at the time of examination is not a true gauge.

disease in the children is at an earlier age than in the parents until, finally, a generation occurs in which the onset is before an age at which the patients mature and the disease ceases to appear in that family.

The phenomenon of increase in severity of disease in succeeding generations has been called "potentiation." For the phenomena of anticipation and potentiation the term "progressive inheritance" has been used. Some authors have denied the existence of anticipation, claiming that it is a statistical error which results from the fact that the material is selected. They say that only those members of earlier generations who live long enough to become parents are considered; whereas, in later generations those who might develop the disease at an older age are not considered. This may be true when one analyzes mass statistics, but is not true, as pointed out by Macklin,[16] in the case of completed pedigrees, such as, for example, was used by Henke and Seeger.[12] The latter suggest three possible explanations for the phenomenon of progressive inheritance: (1) a progressive change in the germinal cell plasm alone, (2) an inherent tendency of the gene conditioning dystrophia myotonica to change continuously and (3) repeated changes in the gene as a result of somatic influences.

On the basis of certain properties of genes which have been determined by recent investigations, we have constructed a hypothetical picture of the process of progressive inheritance. Investigations of Goldschmidt[9] and others indicate that the different behavior of allelomorphs depends, in many if not all cases, on a difference in degree of activity rather than in kind of activity. (See footnote page 599 for definition of allelomorphs.) In the case of many genes a mutation means, therefore, a change to a new level of activity at which the gene usually remains. One would seem justified in assuming that in the case of degenerative disease the gene change may consist of loss of activity. Moreover, this loss of activity is assumed to be of such nature that once begun it progresses slowly but continuously over a period of many years. (See above, Henke and Seeger's second possibility.) Some justification for this latter assumption is found in the observations[9] that in certain plants the changes in the gene are not stable and may occur at various levels in a haphazard manner. The degree of change in the gene in the individual in whom the mutation first appears, and possibly in his children and grandchildren, may be so small that no symptoms or only very mild symptoms occur. Over a period of several generations, however, the activity of the gene is so greatly diminished that although associated with a normal gene, the combined activity is not sufficient for normal function and various degenerative signs appear. In the succeeding generations, the gene activity is further decreased, so the onset of the degenerative change is at an earlier age and more severe in character.

In addition to generational anticipation, some authors speak of a "fraternal anticipation"; that is, the disease appears to have its onset earlier in the younger siblings of a sibship. Although this phenomenon does not appear as regular in its occurrence as the generational anticipation, it is strikingly evident in many of the sibships reported.

Since an approximate 50% incidence of the disease in affected sibships is to be expected with a single dominant factor, we have thus far considered dystrophia myotonica as determined by the presence of a single gene. The occurrence in sibships of cases with muscle symptoms alone, or with cataract alone, or with the two conditions combined has led to the suggestion that we are dealing with linked factors; that is, different genes, each producing some phase of the disease, located in the same chromosome. It is obvious that any dissociation of the signs of the disease that occurs in such case would have to be explained by crossing over; if no crossing over occurred, the linked genes would always occur together and be indistinguishable from a single gene.* That the condition is probably due to a single gene or to several genes so closely linked that crossing over is of very little importance, appears from the following considerations: (1) The vast majority of those affected, if carefully examined and followed over a period of years, sooner or later show all the manifestations of the disease. Many individuals who at the time of their first examination show only one symptom, develop the others later. (2) The complete syndrome may occur in children of parents who show only one of the signs. In Family M. (Fig. 3), for example, H. M. at present has only cataract although his father has myotonia and no cataract (slit lamp examination). (3) If crossing over and consequent dissociation of the symptoms of the disease were very frequent in early generations, the complete syndrome would not occur so frequently and individual symptoms so infrequently in later generations.

The variations in the manifestations of the disease and the special prominence of cataract may be explained as follows: It is now commonly recognized that each gene, although it may most markedly affect one organ or tissue, has an influence in the development of a number of tissues or organs;[2] furthermore, each of the tissues or organs affected by this gene is also affected by numerous other genes. Diminution in the activity of the gene, therefore, affects many tissues. The degree to which each tissue will be affected will depend on the pattern of the other genes affecting that tissue; thus the diversity in the manifestations of the disease. When the action of the gene is only mildly defective, only those tissues will show

* Since genes which exhibit linkage do so because they are located in the same chromosome, a break in the linkage can be caused only by an interchange of parts between members of a pair of homologous chromosomes. The mechanism of interchange of parts is called "crossing over."

TABLE 1.—SUMMARY OF CASES IN WHICH DYSTROPHIA MYOTONICA HAS OCCURRED IN TWO SUCCESSIVE GENERATIONS.

(In the middle columns with the boldface figures the probable age of onset of the disease in parent and children may be contrasted.)

Authors.*	Parents.						Children.					
	Case designation.	Age.†	Sex.	Examined or not examined.	Severity.†	Probable age of onset.‡	Probable age of onset.	Severity.	Case designation.	Age.	Sex.	Examined or not examined.
Curschmann (1912)	J. B.	55	M	E	S	42	21	M	J. E. B.	33	M	E
Grund (1914) .	M. Pf.	47	F	E	L	18	10	M	L. Pf.	28	M	E
							10	S	W. Pf.	20	M	E
Kyrieleis (1925) .	Mother P.	71	F	E	M	45	28	S	P. P.	43	M	E
							20+		Sister P.	39d	F	NE
							30	M	A. P.	36	M	E
Heine (1925) . .	L. W.	43	F	E		34	<19		W. W.	21	M	E
Frey (1925–26) .	G. A.	52d	M	NE	S	30+	14	S	EA.	35	M	E
	M. J.	58	M	E	S	<30	25	S	M. J.	31	M	E
							17	S	F. J.	30	M	E
	G. M. M.	58	M	E		30+	20	S	H. J. M.	23	M	E
Henke and Seeger (1927–28)	E II 7eβ		M	E	L	48	17		E II 7eβl′		M	NE
	E II 7fϵ		F	E	M	34	7-14	S	E II 7fϵl′		M	E
	E III 6α		M	E	L	50?	<26	S	E III 6$\alpha\kappa$		M	E
							40?	L	E III 6$\alpha\eta$		M	E
	F II 10g		F	E	L	<50	<27	S	F II 10gβ		F	E
							<32		F II 10gδ		M	E
							<21	S	F II 10gϵ		M	E
							<18	S	F II 10gη		M	E
	Sch.		F	E	M	35	<18	S			F	E
							<15	S			M	E
							<13				M	NE
Gifford *et al.* (1929)	Mrs. L.	43	F	E	M	33	13	L	L. L.	18	F	E
Bielschowsky *et al.* (1933)		61d	M	E	S	35+	18	S	W. Raf.	41d	M	E
Maas (1937)	S. B. (II)	50	F	E	L	<50	In-fancy	S	SB (III)	26		E
	D (II)	50	M	E	M*	35	<22		D (III) 1	22	M	E
							<19		D (III) 2	19	F	E
	D I	76	F	E	L	<76?	35	M	D (II)	50	M	E
	Mrs. C.	59	F	E	L	40	<26			26		E
	Mrs. G.	48	F	E	L	<46?	<24	L	L	24	M	E
							19	L	V	21	F	E
							<17	L	D	17	F	E
							13	L	H	14	F	E

TABLE 1.—*Continued.*

Maas (1937) (*Continued*)	FTAR	50	M	E	S	30	<28		1				
							<23		3				
							<20		4				
							<18		6				
							<15		7				
							<13		8				
	J. McD.	56	M	E		20	18	S		25	M		
							10	S		17d	M	E	
							8	S		14	M		
	H. D.	53		E		37	<23		Ellen	23	F	E	
	P. B.	53		E		36	16			24	F	E	
	Mrs. C.	43	F	E	L	<43	<19	L		19	F		
	J. G.	55	M	E	S	50	<32	L	Ada	32	F	E	
	P. B.	46	M	E	S	30	7	S	B. B.	18	M		
	Karl S.	53	M	E		<53	<24		W. S.	24	M		
	K. R.	51	F	E	S	<48	<28	L		28	F		
							<21	S		21	M		
	Mrs. A.	55	F	E	S	<31	10	S		29	F	E	
	Mrs. W.	50	F	E	L	<32	<18	S		18	F		
							<13	S		14	M		
	Mrs. D.	58	F	E	L	<52	<24	M		24	F		
							<19	S		19	M		
Ravin and Waring (1938)	J. B.	60	M	E	S	47	18	M	R. B.	27	M	E	
							13	L	M. J. B.	20	F	E	

* Boeters' cases have not been included for the reasons given in the footnote, page 594.
+ "d" after the age indicates that the patient was dead at the time of the report.
† "S" = severe case. "M" = moderately severe case. "L" = light case.
‡ The exact age of onset of this disease is difficult to determine and in many cases the authors have been vague. At best, therefore, the ages given are approximations. The sign "<" has been used to indicate "less than," and "?" has been used when there was some doubt regarding the diagnosis.

involvement in which the other factors are of such a nature as to predispose to the manifestation of the defect. When the gene is markedly defective most of the tissues affected by the gene will show involvement regardless of the nature of the modifying factors. In the case of cataract we may assume a modifying factor, widely distributed, which by itself produces no lens changes, but which will produce cataracts in the presence of a mildly defective dystrophia myotonica gene, too mildly involved to produce other symptoms. If this modifying factor is present, cataract occurs as an early symptom; if the modifying factor is absent, cataract may not occur in fairly marked cases of the disease.

Most studies of sex incidence have shown a preponderance of males but not to a degree suggesting sex linkage. Several of the

more complete studies have shown an equal incidence of the sexes. A preponderance of males in smaller series not completed by family investigations probably results from the following circumstances: (1) Men are more likely to present themselves to a doctor because the disease affects their working ability. (2) If a man is affected, his economic status becomes such that he is likely to be seen in a clinic; whereas, when the woman is affected the economic status of the family is not changed, and she is more likely to be seen by a private doctor.

Certain clinical criteria have been advanced for a group of diseases known as the heredo-degenerative diseases. These criteria are: (1) The disease affects as a rule several members of the same generation in the same manner. This has been called "homologous" heredity. (2) The onset of the disease in the members of the same generation is at about the same age. This "homochromous" heredity is, of course, in contrast with "fraternal anticipation." (3) The basis of the disease is endogenous. (4) The disease progresses irresistibly from the moment of its onset. It is evident that dystrophia myotonica fulfills these criteria and belongs in the group of heredo-degenerative diseases. The explanation of these criteria in terms of mendelian inheritance is also clear.

Families in which such degenerative diseases occur have been thought to show degenerative stigmata; that is, members who do not show the disease may show other stigmata, such as mental or physical inferiority, epilepsy, sterility, and so on, which may be considered degenerative. By comparing the number of such stigmata in a family in which dystrophia myotonica was traced through several generations with the incidence in a comparable normal family, Henke and Seeger[12] came to the conclusion that the incidence of these conditions was not increased in the dystrophic family and that these conditions had nothing to do with the anlage for dystrophia myotonica.

Summary. 1. Dystrophia myotonica, a disease belonging to the heredo-familial group of diseases, affects many tissues and organs of the body and is of interest to workers in many fields of medicine.

2. Four family trees in which the disease occurs are presented. In these family trees, 12 cases of dystrophia myotonica were found among 33 members examined. At least 4 additional cases of the disease occurring in the family could not be investigated.

3. A table is presented in which most instances to be found in the literature of direct transmission of the disease from parent to child are tabulated. This table shows strikingly that in all of these cases the onset of the disease in the children was at a definitely earlier age than in the parents.

4. Evidence leads to the conclusion that dystrophia myotonica

is transmitted as a single factor dominant but that this dominance is modified by the occurrence of "progressive inheritance." In "progressive inheritance" the onset of the disease is at an earlier age in each succeeding generation (anticipation) and the disease increases in severity in succeeding generations (potentiation).

5. Because of the presence of progressive inheritance, on the one hand the parents of many patients may show little or no signs of the disease, and on the other hand the children of patients tend to develop the disease so early as to prevent further propagation and the diseases dies out in the family.

6. Possible changes in the germinal material which could produce this type of heredity are described.

REFERENCES.

(1.) **Barker, L. F.**: Med. Clin. North America, **14**, 109, 1930–31. (2.) **Baur, E., Fischer, E.**, and **Lenz, F.**: Human Heredity, New York, The Macmillan Company, 1931. (3.) **Bielschowsky, M., Maas, O.**, and **Ostertag, B.**: Festschrift f. G. Marinesco, Bucarest, p. 71, 1933. (4.) **Boeters, H.**: Sammlung psychiatrischer und neurologischer Einzeldarstellungen, Leipzig, Georg Thieme, vol. **8**, 1935. (5.) **Curschmann, H.**: (*a*) Deutsch. Ztschr. f. Nervenh., **45**, 161, 1912; (*b*) Deutsch. Arch. f. klin. Med., **149**, 129, 1925. (6.) **Fleischer, B.**: (*a*) Arch. f. Ophth., **96**, 91, 1918; (*b*) Arch. f. Rassen- und Gesellsch. Biol., **14**, 13, 1921–22. (7.) **Frey, H. C.**: Arch. f. Rassen- u. Gesselsch. Biol., **17**, 1, 1925. (8.) **Gifford, S. R., Bennett, A. E.**, and **Fairchild, N. M.**: Arch. Ophth., **1**, 335, 1929. (9.) **Goldschmidt, R.**: Quart. Rev. Biol., **3**, 307, 1928. (10.) **Grund**: München. med. Wchnschr., **60**, 863, 923, 1913. (11.) **Heine, L.**: Ztschr. f. Augenh., **55**, 1, 1925. (12.) **Henke, K.**, and **Seeger, S.**: Ztschr. f. d. ges. Anat., Part II, **13**, 371, 1927. (13.) **Kolb, L. C., Harvey, A. M.**, and **Whitehill, M. R.**: Bull. Johns Hopkins Hosp., **62**, 188, 1938. (14.) **Kyrieleis, W.**: Klin. Monatsbl. f. Augenh., **74**, 404, 1925. (15.) **Maas, O.**: Brain, **60**, 498, 1937. (16.) **Macklin, M. T.**: Human Biol., **4**, 69, 1932. (17.) **Minkowski, M.**, and **Sidler, A.**: Schweiz. med. Wchnschr., **58**, 1005, 1928. (18.) **Nissen, K.**: Verhandl. d. deutsch. Gesellsch. f. inn. Med. Kong., **46**, 108, 1934.

THE CHEMOTHERAPY OF EXPERIMENTAL TYPE II PNEUMOCOCCIC MENINGITIS.

By Paul Gross, M.D.,
PATHOLOGIST, THE WESTERN PENNSYLVANIA HOSPITAL,
AND
Frank B. Cooper, M.S.,
RESEARCH CHEMIST, THE WESTERN PENNSYLVANIA HOSPITAL,
With The Technical Assistance of
Marion Lewis,
PITTSBURGH, PA.

(From The Western Pennsylvania Hospital, Institute of Pathology, Ralph R. Mellon, M.D., Director.)

Following the demonstration of the efficacy of sulphanilamide against experimental pneumococcic infections in mice by Rosenthal[19] and by Cooper, Gross, and Mellon,[7] the latter investigators[5a,b,8,a,b] demonstrated an even greater therapeutic action against experi-

mental pneumonia caused by Types I, II, and III pneumococci in rats. In these experiments, sulphanilamide therapy appeared to be equal to, or better than, optimum serum therapy and the combination of the two more efficacious than either alone against rat pneumonia produced by Types I and II pneumococci. Furthermore, it was shown independently by us[8b] and by Branham and Rosenthal[2] that sulphanilamide and serum acted synergistically in pneumococcic infections. On the basis of these experimental results, it was suggested that sulphanilamide be tried in human pneumonia, either as an adjuvant to serum therapy, or in the absence of serum, as the primary therapeutic agent. The Special Report of the Pneumonia Advisory Committee to the Surgeon General[23] contains a similar recommendation.

In spite of repeated demonstrations on mice, rats and rabbits which showed the effectiveness of sulphanilamide in experimental infections caused by pneumococci of Types I,[2,8b,11,14,19,20,25] II,[5b,6a,19,20] III,[5a,7,8a,19,20] VII,[6b] and XIV,[22] various authors have neglected to mention any type other than Type III as being susceptible to sulphanilamide therapy in either animals or man.

Clinical corroboration of the experimental results was initiated with the report by Heintzelman, Hadley, and Mellon[9] dealing with sulphanilamide therapy of Type III pneumococcic pneumonia. This has been followed during the past year by other publications which tended to demonstrate the efficacy not only of sulphanilamide but also of other sulphonamide derivatives in clinical pneumonia,[1,15] meningitis,[1,10,12,13,16–18,24] and other infections caused by pneumococci.[3,4,21]

Inasmuch as pneumococcic meningitis in man has an extremely high fatality rate,[18] it was thought of interest to obtain data on the susceptibility of experimental pneumococcic meningitis to therapy with sulphanilamide* and 4,4′-di-(acetylamino)-diphenylsulphone.†

In a preliminary publication we[6a] recently indicated that sulphanilamide is very effective in Type II pneumococcic meningitis of rats. In the present report, the detailed findings in a series of 115 rats with Type II pneumococcic meningitis, treated and untreated, are presented.

Method. White rats were anesthetized with ether and a 25-gauge hypodermic needle, 3 mm. long, attached to a tuberculin syringe was pushed to the hilt through the intact skin and skull at a point about 5 mm. lateral to the sagittal suture and midway between eye and ear. The inoculum, consisting of 0.1 cc. of diluted 18-hour broth culture of a Type II pneumococcus (Binda strain), was then slowly injected and any leakage removed with a phenolized swab.

In the following three experimental series, different infecting

* Kindly supplied by E. R. Squibb & Sons, New York.

† Synthesized and donated to us by the Monsanto Chemical Company, St Louis, Missouri.

doses and different periods of delay between infection and the initial treatment were employed.

1. **Experiment With a Moderate Infecting Dose and a 6-hour Delay in the Initial Treatment.** A group of 54 rats was infected with a 10^{-5} dilution of a culture corresponding to about 10 fatal doses, as indicated above. Two rats infected with a 10^{-6} dilution of the same culture died in less than 93 hours. Three hours after the infection, 2 rats were sacrificed and smears made of the heart blood and the lower lumbar portion of the spinal cord. The spinal cord cultures were both positive, whereas only one of the two heart blood cultures was positive. No pneumococci were seen in either of the blood smears or smears of the spinal cord (meninges) although the latter showed numerous neutrophils and some monocytes. Five hours after the infection, or 1 hour prior to the initial treatment, 2 more rats were sacrificed. Both of these gave positive heart blood and positive spinal cord cultures. Smears of the cord (meninges) of both animals showed in addition to numerous neutrophils and some monocytes, also a few pneumococci.

The initial treatments, begun 6 hours after the infection, consisted of oral doses of 100 mg. of sulphanilamide as a 20% suspension in 15% aqueous gum acacia for 15 animals. Doses of 100 mg. of 4,4′-di-(acetylamino)-diphenylsulphone, similarly suspended, were administered to 15 other rats. The remaining 20 animals were not treated and served as controls. These doses were given twice daily for 2 days and then once daily for 8 subsequent days.

All survivors were sacrificed 22 days after infection and cultures and smears made of the heart blood and distal portion of the spinal cord (meninges). The spine with attached skull was removed and fixed in formalin, after which the brain and cord were carefully dissected and sections, stained with hematoxylin and eosin, were made. Similar procedures were followed with all the fatalities.

Results. The results are shown graphically in Figure 1. While the fatality rate in the control group was 100% and 60% in the sulphone group, the sulphanilamide-treated group showed only a 26.7% fatality. Similar differences were found in the average survival time of the fatalities which was 36½ hours for the control group, 68 hours for the sulphone group, and 8½ days for the sulphanilamide group.

In the control group all blood and spinal cord cultures were positive. Of the fatalities in the sulphone group, all blood cultures and all but one of the spinal cord cultures were positive, while of the 4 sulphanilamide treated fatalities, 3 showed positive blood cultures, and only 1 a positive spinal cord culture. All survivors gave negative blood and spinal cord cultures.

2. **Experiment With a Heavy Infecting Dose and a 6-hour Delay in the Initial Treatment.** A series of 45 rats was infected with a 10^{-4} dilution of the same culture as in Experiment 1, using the same

technique. This dilution corresponded to about 100 fatal doses. Treatments identical with those of the preceding experiment were initiated 6 hours following the infection and continued daily for 10 days, with 2 daily treatments on the first 2 days. There were 15 rats each in the control, the sulphone, and the sulphanilamide groups. Cultures and smears of the heart blood and spinal cord, as well as sections of the brain and the spinal cord were made of all fatalities and of the survivors which were sacrificed after 22 days.

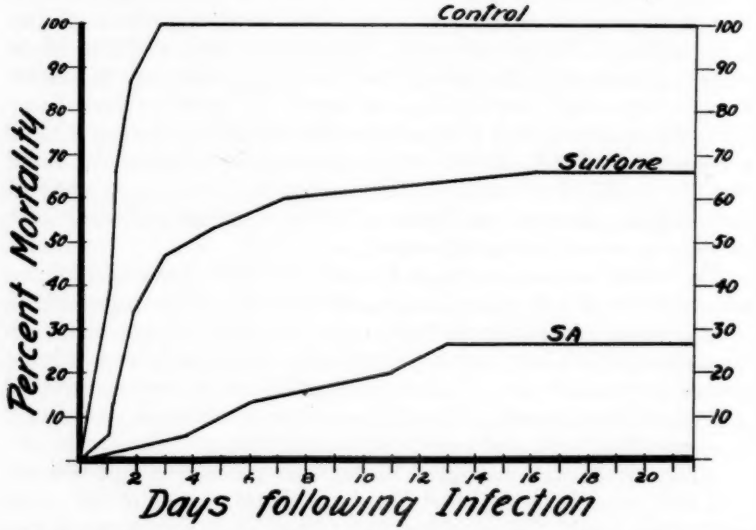

FIG. 1.—Mortality rate of rats infected intracranially with Type II pneumococci (10 fatal doses). All rats were infected with 0.1 cc. of an 18-hour broth culture, diluted 10^{-5}, of Type II pneumococcus (Binda strain). All, except control animals, were treated 6 hours following infection, then twice daily for 2 days, and subsequently once daily for 8 more days. Dose at each treatment was 100 mg. by mouth. Control group consisted of 20 rats; sulphone group 15 rats; SA (sulphanilamide) group 15 rats. All surviving animals were sacrificed on the 22d day.

Results. The results are shown graphically in Figure 2. All controls were dead within 68 hours and all but 1 of the sulphone-treated rats within 44 hours. This animal survived to the end of the experiment. The sulphanilamide group on the other hand showed a 46.7% survival rate with an average survival time of 133 hours for the fatalities.

All blood and spinal cord cultures of the control group were positive. Among the fatalities of the sulphone group there were 1 negative blood and 1 negative spinal cord culture, while from the sulphanilamide-treated fatalities 3 negative blood and 4 negative spinal cord cultures were obtained. All survivors, 7 in the sulphanilamide group and 1 in the sulphone group, gave negative blood and spinal cord cultures.

3. **Experiment With a Moderate Infecting Dose and a 12-hour Delay in the Initial Treatment.** A group of 14 rats were inoculated with about 10 fatal doses as in the first experiment. Seven of the rats were not treated and served as controls, while the other animals were given initial treatments 12 hours after the infection. These treatments consisted of 100 mg. of sulphanilamide by mouth twice daily for 5 days and then once daily for 9 more days. The survivors were sacrificed after 4 weeks.

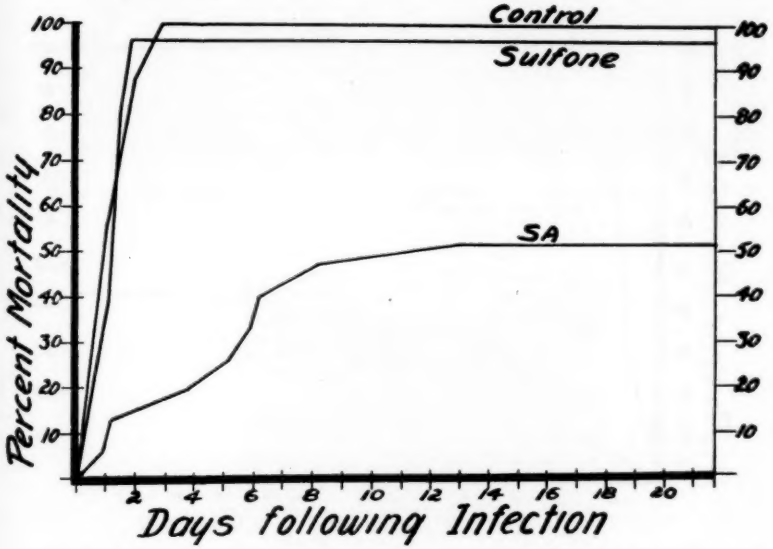

FIG. 2.—Mortality rate of rats infected intracranially with Type II pneumococci (100 fatal doses). All rats were infected with 0.1 cc. of an 18-hour broth culture, diluted 10^{-4}, of Type II pneumococcus (Binda strain). All, except control animals, were treated 6 hours following infection, then twice daily for 2 days, and subsequently once daily for 8 more days. Dose at each treatment was 100 mg. by mouth. Each group consisted of 15 rats. All surviving animals were sacrificed on the 22d day. SA—sulphanilamide.

Results. All untreated rats died within 123 hours (Fig. 3) with an average survival time of 60 hours, while only 3 of the 7 treated animals died (43%), and these with an average survival period of 9.4 days. The last fatality in the treated group occurred on the 19th day. This animal, which had a negative spinal cord but a positive blood culture, died of exsanguination from a ruptured, septic, enlarged spleen.

Symptomatology. The symptoms displayed by the infected rats were variable. While many of the animals merely became weak, lay on one side, and refused to feed until death supervened, others showed incoördination, athetoid movements of the head, loss of equilibrium, and marked irritability. Several rats developed con-

vulsions, a few squealed, and several others, when touched, began biting cage mates indiscriminately. Many of the rats developed wet or crusted noses and crusting about the eyes, giving to the latter a mascara-like effect. Although many of the rats exhibiting convulsions, ataxia, and other disturbances died, there were some that recovered under treatment.

Pathologic Studies. Grossly, only occasional animals showed slight subpial ecchymosis in the region of the inoculation. The purulent nature of the meninges was not discernible macroscopically.

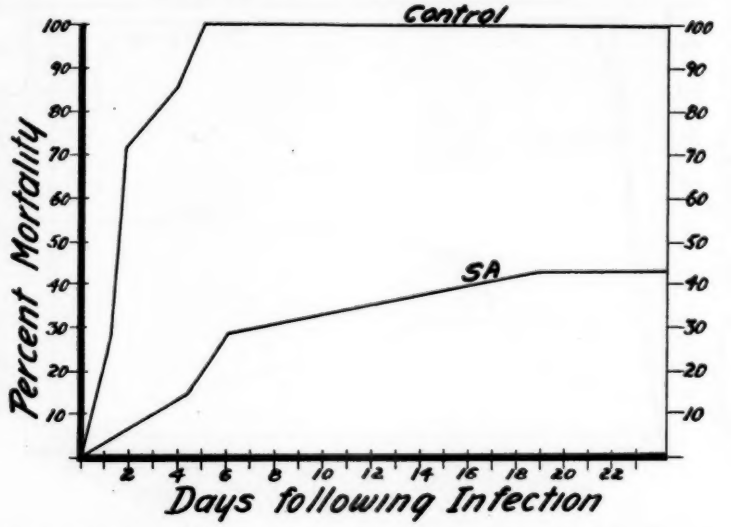

FIG. 3.—Mortality rate of rats infected intracranially with Type II pneumococci (10 fatal doses). All rats were infected with 0.1 cc. of an 18-hour broth culture, diluted 10^{-4}, of Type II pneumococcus (Binda strain). Control group of 7 rats were untreated. SA (sulphanilamide) group of 7 rats were treated 12 hours following infection, then twice daily for 5 days, and subsequently once daily for 9 more days. Dose at each treatment was 100 mg. by mouth. Survivors were sacrificed after 4 weeks.

Microscopically, within 3 hours after infection there were a few foci in various regions of the brain characterized by subpial infiltration of some neutrophils, a few monocytes, and red blood cells. Similar but smaller foci were found at various levels of the spinal cord. Sections from 2 rats killed 5 hours after infection showed no further progress of the inflammation.

Within less than 20 hours 6 control and 3 sulphone-treated rats had died and these, as well as others subsequently, showed extensive and diffuse, although not a uniformly distributed purulent leptomeningitis of the brain (Fig. 4) and spinal cord (Fig. 5). In some animals, the subpial cortex also showed leukocytic infiltration with associated degeneration and necrosis of the brain tissue.

FIG. 4.—Meningitis of brain showing extension of purulent exudate deep into the sulci. Control rat dead in 26 hours. (H. and E. × 57.)

FIG. 5.—Spinal cord showing the purulent meningitis and purulent infiltration of cord substance. Control rat dead in less than 44 hours. (H. and E. × 70.)

FIG 6.—Central canal of cord, filled with pus, showing ulceration of ependyma and beginning infiltration of adjoining cord substance. Sulphone-treated rat dead in 29 hours. (H. and E. × 170.)

FIG. 7.—Cortex showing large focus of meningeal calcification with gliosis of underlying cortex and a collection of hemosiderin containing phagocytes. Sulphanilamide-treated rat sacrificed 4 weeks after infection. (H. and E. × 140.)

Chemotherapy resulted in significant differences in incidence and severity of lesions which are summarized in Table 1. About 67.5%

TABLE 1.—INCIDENCE AND SEVERITY OF LESIONS FOUND IN THE DIFFERENT INFECTED GROUPS.*

	Control groups.		Sulphone-treated groups.		Sulphanilamide-treated groups.	
Type of lesion.	No. animals.	%	No. animals.	%	No. animals.	%
Meningitis of brain, slight involvement	6	16.2	16	53.3	31	83.7
Meningitis of brain moderate to severe involvement	29	78.3	12	40.0	1	2.7
Pus in cerebral ventricles	25	67.5	16	53.3	2	5.4
Encephalitis	21	56.7	15	50.0	5	16.7
Spinal meningitis slight involvement	8	21.6	3	10.0	5	13.5
Spinal meningitis moderate to severe involvement	25	67.5	9	30.0	0	0.0
Pus in central canal of spinal cord	17	45.9	5	16.7	0	0.0
Myelitis	16	43.2	12	40.0	1	2.7
Death	37	100.0	23	76.7	15	40.5

* These groups represent animals combined from the various experiments.

of the untreated, 53.3% of the sulphone-treated, and 5.4% of the sulphanilamide-treated rats showed pus in the cerebral ventricles, frequently associated with focal ulceration of the ependymal lining and extension of the leukocytic infiltration into the adjoining brain tissue. A few control as well as treated animals exhibited multiple small cerebral abscesses. The leukocytic infiltration of the brain substance was commonly associated with multiple small and larger hemorrhages.

Pus in the central canal (Fig. 6), often associated with purulent myelitis (Fig. 5), was present in 45.9% of the untreated, 16.7% of the sulphone-treated, and none of the sulphanilamide-treated animals. Rats that died 5 or more days following the infection, or those that were sacrificed at the conclusion of the experiments, showed in the pia a scanty but focally often more abundant monocytic infiltration associated with slight thickening and basophilia of the pia, as well as swelling and proliferation of the pial cells. Indications of healed or healing more destructive lesions were found in some of the recovered animals.* These consisted of large calcium deposits in the meninges (Fig. 7) as well as in the brain tissue, and hemosiderin deposits in the subpial cortex as well as in the paraventricular regions. Indications of a previously existing meningitis could be found in only a few of the spinal cords which showed a scanty monocytic infiltration about some of the larger pial vessels.

Discussion. Although smears, cultures and sections of the spinal cords demonstrated the existence of pneumococcic meningitis at

* A more detailed study of these lesions will be reported elsewhere.

least 3 hours prior to treatment, it is probable that therapy arrested the disease before it reached the proportions usually encountered in man. Nevertheless, all the recovered rats showed evidence of meningitis and some showed more deeply seated lesions (scars, hemosiderin and calcium deposits), which were healed or healing.

Therapy with 4,4′-di-(acetylamino)-diphenylsulphone proved inferior to that with sulphanilamide. This is in agreement with similar findings previously reported by us.[6b] The fatality rate of the sulphone-treated animals was almost double that of the combined sulphanilamide-treated groups (Table 1). Similarly, the incidence and severity of lesions in the sulphanilamide-treated groups (fatalities as well as survivals) was fractional compared with those of the control or sulphone-treated groups.

The Type II pneumococcus was chosen for these experiments in order to dispel the mistaken notion that only Type III pneumococcic infections are susceptible to sulphanilamide therapy. It should be borne in mind, however, that although sulphanilamide therapy has been proven effective in experimental pneumococcic pneumonia,[5a,b,8a] clinical corroboration is as yet very incomplete. On the other hand, it has been demonstrated experimentally by us,[8b] as well as by Branham and Rosenthal[2] that sulphanilamide and antipneumococcic serum act synergistically. In addition, more recent work[8c] with Type I pneumococcic meningitis of rats has shown that a new concentrated antipneumococcic rabbit serum* gave results superior to those obtained with sulphanilamide, and that both together gave better results than either alone.

It therefore appears probable that sulphanilamide administered as an adjuvant to serum therapy or alone when the appropriate antipneumococcic serum is not available, may play an important rôle in lowering the mortality rate of pneumococcic pneumonia and meningitis in man.

Conclusions. 1. A cerebrospinal meningitis has been produced in rats with a Type II pneumococcus.

2. Sulphanilamide therapy of this disease effected a marked reduction in mortality as well as in incidence and severity of the lesions.

3. Therapy with 4,4′-di-(acetylamino)-diphenylsulphone was less effective against this infection than sulphanilamide.

4. Sulphanilamide is suggested as an adjuvant to specific serum therapy, or as the primary therapeutic agent when such serum is not available, as a means toward an effectively lowered mortality rate of pneumococcic pneumonia and meningitis in man.

REFERENCES.

(1.) **Basman, J.**, and **Perley, A. M.**: J. Pediat., **11**, 212, 1937. (2.) **Branham, S. E.**, and **Rosenthal, S. M.**: Pub. Health Rep., **52**, 685, 1937. (3.) **Brown, A. E.**, and **Bannick, E. G.**: Proc. Staff Meet., Mayo Clinic, **12**, 644, 1937. (4.) **Cain, A.**, **Cattant, R.**, and **Sikovay, H.**: Presse méd., **46**, 577, 1938. (5.) **Cooper, F. B.**, and

* Kindly supplied by E. R. Squibb & Sons, New York.

Gross, P.: (*a*) Proc. Soc. Exp. Biol. and Med., **36**, 678, 1937; (*b*) Ibid., p. 774. (**6.**) **Cooper, F. B., Gross, P.**, and **Lewis, M.**: (*a*) Ibid., **38**, 835, 1938; (*b*) Am. J. Med. Sci., **196**, 343, 1938. (**7.**) **Cooper, F. B., Gross, P.**, and **Mellon, R. R.**: Proc. Soc. Exp. Biol. and Med., **36**, 148, 1937. (**8.**) **Gross, P.**, and **Cooper, F. B.**: (*a*) Ibid., p. 225; (*b*) Ibid., p. 535; (*c*) Therapeusis of Experimental Type I Pneumococci Meningitis in Rats, etc., Am. J. Med. Sci. (in press). (**9.**) **Heintzelman, J. H. L., Hadley, P. B.**, and **Mellon, R. R.**: Am. J. Med. Sci., **193**, 759, 1937. (**10.**) **Hubert, C.**: Presse méd., **46**, 771, 1938. (**11.**) **Kreidler, W. A.**: Proc. Soc. Exp. Biol. and Med., **37**, 146, 1937. (**12.**) **Landon, J.**: Brit. Med. J., **1**, 844, 1938. (**13.**) **Latto, C.**: Ibid., p. 566. (**14.**) **Locke, A., Locke, R. B., Bragdon, R. J.**, and **Mellon, R. R.**: Science, **86**, 228, 1937. (**15.**) **Louis, D. J.**: Illinois Med. J., **73**, 422, 1938. (**16.**) **Martin, R.**: Presse méd., **46**, 599, 1938. (**17.**) **Mertins, P. S.**, and **Mertins, P. S., Jr.**: Arch. Otol., **25**, 657, 1938. (**18.**) **Neal, J. B.**, and **Appelbaum, E.**: Am. J. Med. Sci., **195**, 175, 1937. (**19.**) **Rosenthal, S. M.**: Pub. Health Rep., **52**, 48, 1937. (**20.**) **Rosenthal, S. M., Bauer, H.**, and **Branham, S. E.**: Ibid., p. 662. (**21.**) **Sager, W. W.**, and **Raffel, W.**: Med. Ann. Dist. of Columbia, **7**, 99, 1938. (**22.**) **Schmidt, L. H.**: Proc. Soc. Exp. Biol. and Med., **37**, 205, 1937. (**23.**) Special Report of Pneumonia Advisory Committee to the Surgeon General, U. S. Pub. Health Serv., J. Am. Med. Assn., **110**, 1701, 1938. (**24.**) **Tixier, L., Eck, M.**, and **Grossiard, —.**: Presse méd., **46**, 599, 1938. (**25.**) **Whitby, L. E. H.**: Lancet, **1**, 1517, 1937.

LYMPHOCYTIC CHORIOMENINGITIS.

Report of a Fatal Case With Autopsy Findings.

By T. E. Machella, M.D.,

Edward W. Bok Fellow in Clinical Medicine; Hospital of the University of Pennsylvania.

L. M. Weinberger, M.D.,

Charles Harrison Frazier Fellow in Neurosurgery; Hospital of the University of Pennsylvania.

and

S. W. Lippincott, M.D.,

Resident in Pathology; Hospital of the University of Pennsylvania, Philadelphia, Pa.

(From the Department of Medicine and the Neuropathological Laboratories of the Hospital of the University of Pennsylvania, and the Department of Pathology, School of Medicine, University of Pennsylvania.)

During the past several years there has come to be recognized a clinical entity which has been labelled under various headings: acute aseptic meningitis,[11] lymphocytic meningitis,[6] acute lymphocytic choriomeningitis[1] and benign lymphocytic meningitis.[4] With the exception of the fatal case reported by Viets and Warren[10] and the case which developed an obliterative arachnoiditis of the spinal cord, reported by Barker and Ford,[3] all cases reported in the literature have run a benign course, a few more protracted than others. The clinical picture has become established, and a responsible virus has been identified by Rivers and Scott[9] and by Findlay, Alcock and Stern.[5] Antibodies have been demonstrated by Armstrong and Dickens.[1] However, as 11% of cases whose serum was examined by Armstrong and Wooley[2] for the presence of protective antibodies gave positive results without recognizable disease of the central

nervous system, the question arises whether or not the clinical entity of "aseptic meningitis" is made up of more than one etiologic entity. Tuberculosis, encephalitis and non-paralytic poliomyelitis in certain respects simulate the clinical findings. A case diagnosed on clinical grounds as benign lymphocytic meningitis by Viets and Warren[10] at necropsy showed evidence of encephalitis. It is the purpose of this paper to report another fatal case with necropsy findings, whose clinical course was that of lymphocytic choriomeningitis.

Case Report.—G. T. M., white male, aged 14 was admitted to the service of Dr. O. H. P. Pepper in the University Hospital on February 4, 1938, and died on February 24, 1938. Ten days prior to admission a dull frontal headache associated with sneezing developed. The next day there was photophobia, anorexia, weakness and listlessness. On the third day, a severe shaking chill was experienced; on the fourth, sudden nausea with repeated bouts of forceful vomiting. Headache disappeared on the seventh day and it was noted that the patient appeared warm, flushed and perspired freely. The 5 days prior to admission were spent in bed chiefly in one position; this consisted of lying on the side with thighs flexed, neck rigid, head extended backwards, pain resulting on attempting to bring the chin forward. He was admitted on the tenth day of the disease, vomiting everything ingested, including fluids taken in an attempt to allay a severe thirst. Past medical history was negative except for whooping cough at 7, chicken pox at 8 and measles at 12 years of age. Family history contained no known tuberculosis.

Physical Examination.—On admission the boy was oriented, quiet, apathetic and drowsy. Temperature was 101, pulse 120 and respiration rate 20 per minute, blood pressure 120/70 (r). There was cervical rigidity, positive Brudzinski's and Kernig's signs and bilaterally equally hypoactive biceps, triceps, Achilles and patellar reflexes. Eyes and eyegrounds were negative except for slightly blurred disc margins. Abdominal reflexes were active. There was no Babinski, Gordon, or Hoffman sign, patellar nor

---

LEGENDS FOR FIGS. 1 TO 6.

FIG. 1.—The meningeal reaction over the cortex is characterized by thickening due to heavy connective tissue proliferation. There is only a sparse scattering of inflammatory cells within the meshes. Note that the infiltrations are heavier in the sulci. The vessels are dilated with blood. (H. and E., × 30.)

FIG. 2.—The basal meninges show a far greater degree of infiltration with conglomerate masses of lymphocytes. The subarachnoid spaces are obliterated by the inflammatory and fibroblastic proliferation. (H. and E., × 100.)

FIG. 3.—Portion of the wall of the lateral ventricle, showing complete stripping and fragmentation of the ependymal lining. The lymphocytic perivascular infiltration noted in the subependymal zone is confined to this area. The glial proliferation forms small irregular nodules which project into the ventricle. These are seen at the left of the illustration. (H. and E., × 40.)

FIG. 4.—Portions of the choroid plexus are destroyed and replaced by masses of inflammatory exudate consisting of lymphocytes, red blood cells, desquamated choroidal epithelium and fibrin. Other parts of the plexus though relatively preserved show fragmentation of the epithelial cells. (H. and E., × 30.)

FIG. 5.—The ependyma of the floor of the fourth ventricle is entirely denuded and the ventricular floor is frayed and fragmented. The perivascular infiltration is still confined to the subependymal zone. The cells in the subependymal area are largely swollen astrocytes. (H. and E., × 100.)

FIG. 6.—Section through basal ganglia and lateral ventricles disclose no evidence of demyelinization (Pal-Weigert stain).

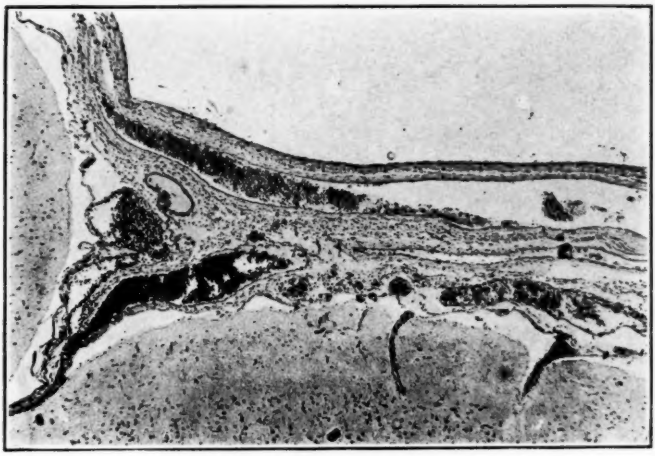

FIG. 1

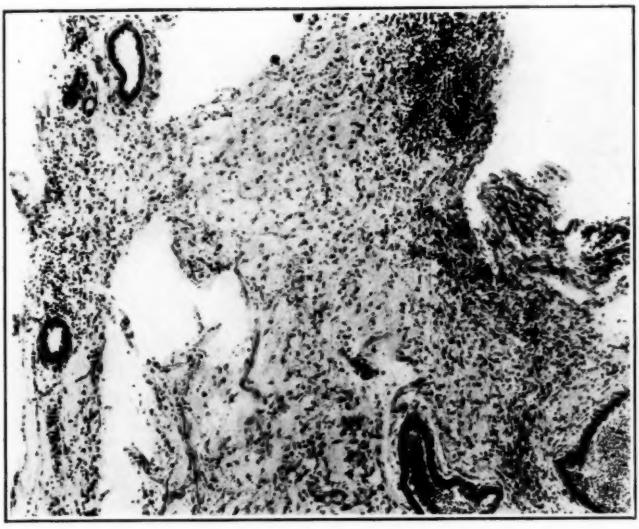

FIG. 2

FIG. 3

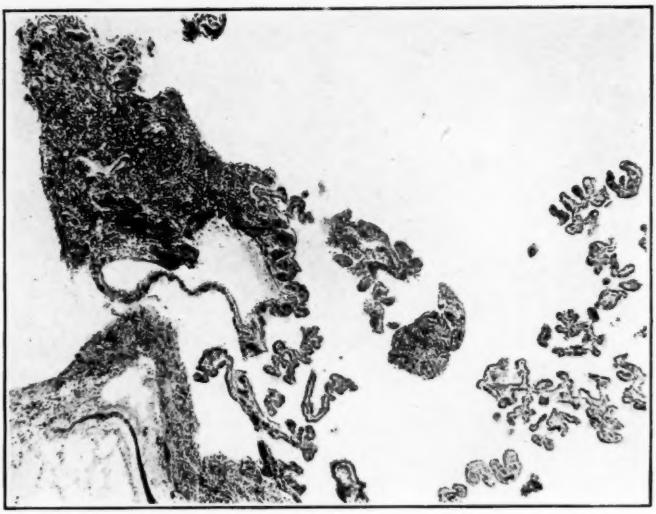

FIG. 4

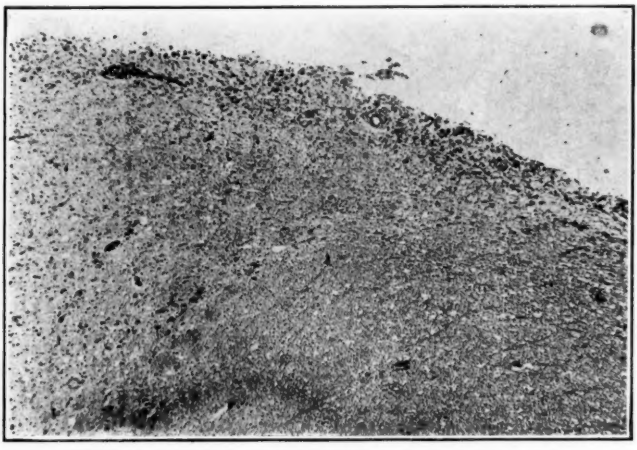

FIG. 5

FIG. 6

ankle clonus. There was no paralysis and speech was normal. The rest of the examination was negative except for a warm, dry skin, heavily coated tongue, engorged mucosa of the turbinates anteriorly, soft blowing systolic basal murmur, tense but not tender abdominal wall.

Laboratory Data.—The white corpuscles numbered 9360, 79% of which were neutrophils. Lumbar puncture performed ½ hour after admission revealed a cloudy fluid, a pressure of 120 mm. of water, 556 cells per c.mm., all of which were lymphocytes (Table 1). Smears for pathogenic bacteria by Gram's method and Ziehl-Neelsen's for acidfast bacilli were negative. Cultures on routine media and on special media for tubercle bacilli were negative. The Kolmer complement fixation test was negative; the colloidal gold curve was 0000111100. For the chemistry of the fluid and characteristics of fluids obtained on subsequent taps see Table 1. Blood Kolmer and Kahn tests and blood cultures were negative. Two guinea pigs were inoculated with 1 cc. of sediment from the fluid, 1 pig received the injection subcutaneously in the groin, the second intraperitoneally. Both pigs died on the 16th day after inoculation; no evidence of tuberculosis was found at necropsy. Congestion of the pigs' lungs was evident and the spleen was slightly enlarged and bloody. The lungs and spleen were ground in salt solution filtered and reinjected into a single pig intraperitoneally. This pig died on the 17th day after inoculation; the organs showed the same gross pathologic picture as outlined above. There was no evidence of virus infection among the guinea pigs nor were there any deaths of a similar nature among other guinea pigs injected.

TABLE 1.—SPINAL FLUID STUDIES.

Date. (1938.)	Day of disease.	Initial pressure in mm. H_2O.	Color of fluid.	Cells.	Percentage lymphocytes.	Protein in mg. %	Sugar in mg. %	Chlorides in Milliequiv. per. liter
2– 4	10	120	Cloudy	556	100	129	49	103
2– 5	11	150	Cloudy	425	100	227		
2– 6	12	140	Cloudy	830	...	297	64	108
2– 7	13	160	Cloudy	910	85	440	45	112
2– 8	14	250	Yellow	710	90	400	59	111.7
2–10	16	220	Yellow	975	90	520	53	111.9
2–15	21	120	Yellow	475	90	401		
2–22	28	90	Yellow	217	100	367		
2–24	30	0	..	63	100	245		

2– 4 Culture and Wassermann test negative. Colloidal gold: 0000111100.
2– 7 No pellicle. Culture for Tb. negative. Guinea pig inoculation for Tb. negative.
2–22 Queckenstedt test negative.
2–24 Queckenstedt test positive. Evidence of block by spinal and cisternal puncture. Rise in spinal pressure of 12 mm. on abdominal straining.

Course in Hospital. In the hospital the boy improved subjectively and objectively, so that by the 6th day after admission (15th day of the disease) temperature was normal, he was more alert, appetite had improved and vomiting and headache had disappeared. Cervical rigidity had markedly decreased but did not disappear. Spinal fluid examinations as in Table 1. On the 21st day of the disease he vomited twice; examination of the eyegrounds showed no detectable changes and spinal fluid pressure was normal. A chest Roentgen ray revealed no evidence of pulmonary tuberculosis. On the 28th day of the disease he developed nausea and vomiting and complained of marked vertigo when he moved his head. Physical examination at this time disclosed a coarse lateral and upward nystagmus and no other findings. The new symptoms were interpreted as being due to an inflammatory invasion or reaction of the membranous labyrinth. There was no

febrile response. The next day the symptoms continued despite the intravenous administration of ammonium chloride in 5% glucose in an attempt to replace sodium ions. On the 29th day his pulse which was averaging about 76 dropped to 52 per minute. Nausea and vertigo persisted and he began to complain of severe headache. Eyegrounds showed the discs to be as before. A lumbar puncture was done in the morning and pressure was zero, the Queckenstedt test was positive. A cisternal tap was done in the afternoon, the pressure again was zero, air was injected with the idea of breaking up adhesions; this procedure was followed by relief from headache and vertigo but no objective improvement, the bradycardia persisting. The next afternoon respiration suddenly ceased and did not resume despite artificial and chemical stimulation and the use of a respirator. The pulse gradually becoming weaker until it faded away.

Necropsy. (17 hours after death.) All organs were examined and with exception of the brain, disclosed no evidence of disease grossly or microscopically. No evidence of tuberculosis was found. The brain weighed 1290 gm. Of the central nervous system, only the brain and upper spinal cord were available for study.

Gross Description of the Brain. The brain (fixed in 10% formalin), showed nothing remarkable in the general appearance of the cortex. The meninges appeared somewhat thickened and this was most noticeable at the base and around the brain stem.

The brain was sectioned coronally. The entire ventricular system was dilated to about twice normal size. There were numerous punctate hemorrhages scattered thickly in the periventricular zones. There were also a few noticeable in the ependyma of the lateral ventricles. The choroid plexuses of the lateral third and fourth ventricles appeared discolored and "beefy." There was a distinct impression of the foraminal ring on the cerebellar tonsils.

Microscopic Anatomy. Routine sections were taken from the frontal, parietal and occipital lobes, the basal ganglia, mid-brain, pons, medulla and upper cervical cord. Paraffin, celloidin and frozen sections were prepared. These were stained with Hematoxylin and Eosin, Hematoxylin and Phloxin, Weil's modification of the Pal-Weigert, Globus's modification of Cajal's gold sublimate technique, Scharlach R and Giemsa and Azure II for inclusion bodies.

Meninges. The meninges over the cortex showed a marked degree of thickening due to fibroblastic proliferation (Fig. 1). Small lymphocytes were scattered thinly through the interstices of the heavy strands of connective tissue. In the sulci the infiltration of inflammatory cells was much heavier. The pial vessels were distended and many of them had ruptured. The fact that numerous macrophages contained blood pigment indicates that these extravasations occurred during life.

The character of the meningeal reaction was different at the base and surrounding the brain stem and cerebellum from that on the cortex (Fig. 2). In these sites the inflammatory reaction was more intense. The meningeal spaces were occupied by thick masses of lymphocytes, polyblastic cells, fibrin and fibroblasts. The marked proliferation of connective tissue cells and their tendency to organization suggests that the reaction was of some duration. In many areas the subarachnoid spaces were obliterated by the masses of organizing exudate. The connective tissue response seemed disproportionate to the inflammatory infiltration. Neutrophils were not seen, neither were plasma cells. There was also much hemorrhage in the leptomeningeal spaces and many macrophages contained pigment.

Cortex. A number of sections of various portions of the cerebral cortex were examined and in general they showed strikingly little. There was a mild degree of pericellular and perivascular edema but these changes may

well have been due to fixation. The ganglion cells for the most part appeared entirely normal. There was a mild diffuse increase in the glial nuclei throughout the cortex and subcortex but this again did not appear striking. The vessels were normal and a thorough search failed to disclose any with perivascular infiltrations. The myelin sheath stains failed to show any evidence of myelin damage in either the cortex or subcortex. No inclusion bodies were found in the ganglion cells.

Basal Ganglia and Ventricles. Blocks were taken through the basal ganglia, lateral and third ventricles. The most obvious and remarkable changes were noted in the ventricular walls. Except for small scattered areas, the ependymal lining of the ventricles was entirely denuded and fragmented (Fig. 3). This was true of the entire ventricular system including the aqueduct and fourth ventricle. Throughout the entire ventricular system the subependymal zone was the site of an intense inflammatory reaction. The subependymal vessels were widely dilated with blood and surrounded by thick cuffs of lymphocytes. The tissue for a depth of 1 to 2 mm. was spongy, pale, frayed and occupied by dense accumulations of swollen glial nuclei. These were for the most part astrocytic, but there was a liberal sprinkling of oligodendroglial cells. Throughout this zone there was also a thin sprinkling of lymphocytes which had apparently wandered out into the tissues from the perivascular spaces. Here and there dense nodules of glial nuclei were noted. With Cajal's gold sublimate method a thick felt-work of glial fibers could be demonstrated in the subependymal zones. The absence of fat with the Scharlach R stain indicated that the inflammatory process was productive rather than necrotizing. At many points in the ventricular walls the dense accumulations of glial cells and fibers formed small microscopic nodules projecting into the ventricles, a so-called "granular ependymitis." These ependymal granulations were scattered throughout the walls of the entire ventricular system. None was seen in the aqueduct large enough to cause obstruction. The presence of this type of ependymitis also indicates that the inflammation was subacute. The septum pellucidum was studded with perivascular infiltrations, distended veins and perivascular hemorrhages. The most interesting observation was the sharp limitation of the inflammation to the area subjacent to the ependyma.

Of all parts of the brain examined, the choroid plexuses of the lateral ventricles showed the greatest degree of inflammatory response. The choroidal vessels were widely dilated and there were numerous hemorrhages within the papillæ. The epithelial cells were swollen and many of the tufts were fragmented and necrotic. In certain areas the structure of the plexus was no longer recognizable and all that one could see was a nodular conglomerate of inflammatory cells, macrophages, red blood cells, fibrin and necrotic epithelial cells (Fig. 4).

Again in the subependymal zone beneath the attachment of the choroid plexus to the wall of the ventricle the inflammatory reaction was very intense, the tissue was pale and edematous and lymphocytes were diffusely scattered through the interstices along with numerous glial cells.

Unfortunately, the choroid plexuses of the third and fourth ventricles were not included in the available sections, so that one can not state what they showed; but it is probable that similar findings existed. Aside from the changes described subjacent to the ependyma, the basal ganglia were essentially normal. The ganglion cells of the thalami and lenticular nuclei showed no degenerative changes. In those portions near the subependymal strip there was a definite increase in the glia but further away there was no significant glial reaction. A search through the basal ganglia failed to disclose any perivasuclar infiltrations. Myelin sheath stains showed no evidence of demyelinization in either the basal ganglia or the internal capsule (Fig. 6). No pathologic changes were noted in the optic nerves.

Midbrain. The ependyma of the aqueduct showed essentially the same changes as were seen in the lateral ventricles. It was stripped and fragmented, the area subjacent was spongy and filled with swollen glial nuclei and lymphocytes. There were large perivascular infiltrations. The ganglion cells of the periaqueductal gray matter were swollen, the Nissl substance was disintegrated and the nuclei were eccentrically placed. The cells of the trochlear and oculomotor nuclei also showed degenerative changes of the same nature. There was a marked increase in the astrocytic glia in the periaqueductal gray substance, and inflammatory cuffs about the vessels. Cells in the deeper nuclei of the reticular substance were normal and myelin stains showed no injury of the tracts and fibers of the midbrain. No inclusion bodies were found in this region.

Pons. The walls, roof and floor of the upper part of the fourth ventricle showed the same pathologic alterations noted in the other portions of the ventricular system. There were numerous ependymal granulations on the floor and walls with the same characteristics as those described in the lateral ventricles., The tegmentum and basilar portions of the pons were altogether normal.

Medulla. The section through the medulla passed through the level of the superior olives. The inflammatory reaction in the floor of the fourth ventricle was very severe. The ventricular floor, as well as the ependyma, was disintegrated to a depth of about 1 mm. The tissue was packed with lymphocytes and large ameboid glial cells. This was the only area where the reaction was severe enough to cause inflammatory softening. The region of the acoustic tubercles on both sides and the medial vestibular nucleus on the left were necrotic and filled with "gitter" cells, proliferated and degenerated astrocytes, tissue fragments and lymphocytes. The vessels in these regions were surrounded with wide perivascular cuffs and the veins were engorged with blood. The ganglion cells of the *substantia grisea reticularis* were shrunken and pyknotic. The nerve cells of the superior olives showed degenerative changes. In spite of these ganglion cell alterations in the deeper portions of the medulla there were no perivascular infiltrations except in the subependymal zone and the area of infiltration was sharply limited to the region of the ventricle. The evidences of injury to the deeper gray masses point to the penetration at least of toxic substances. In general, the inflammatory reaction seemed to extend deeper beneath the ependyma of the fourth ventricle than elsewhere in the ventricular system (Fig. 5).

Cerebellum. The nuclei of the cerebellum were entirely normal. This was also true of the cortex and subcortex of the cerebellum. No perivascular infiltrations were found in this structure.

The ependyma of the central canal was present and intact. The ganglion cells of the anterior horns were entirely normal. There was no evidence of demyelinization. The meninges surrounding the cord were thickened and were sparsely infiltrated with lymphocytes. There were also small extravasations of blood from the distended veins.

Pathologic Summary. In brief, the essential pathologic findings consisted of a marked thickening of the meninges due to connective tissue proliferations with obliteration of the subarachnoid spaces. The meninges were infiltrated with lymphocytes, red blood cells and macrophages. The cortex, basal ganglia and subcortical white matter were on the whole unaffected. The ependyma of the entire ventricular system was denuded and fragmented and a narrow subependymal zone was the site of an intense inflammatory reaction characterized by perivascular lymphocytic infiltrations, glial proliferations, engorgement of the vessels and hemorrhages. The walls of the ventricles were studded with ependymal granulations. The choroid plexuses were partly necrotic and heavily infiltrated with inflammatory exudate. In the floor of the fourth ventricle the process was severe enough to cause softening especially involving the acoustic tubercles and

the right medial vestibular nucleus. The disease was unusual in that while the meninges, ventricular walls and choroid plexuses showed an intense inflammatory reaction, the remainder of the brain substance showed none at all and was essentially normal.

Pathological Comment.—The cause of death in this patient appears to have been due to increased intracranial pressure, a result of block caused by inflammatory obliteration of the subarachnoid fluid spaces. This is suggested by the fact that death was sudden and that it coincided with the finding of block by lumbar and cisternal puncture. This impression is substantiated by the presence of dilated ventricles, clear evidence of a preëxisting interference with the C.S.F. circulation and by the presence of a pressure cone. The correlation of the patient's symptoms with the pathologic findings in the brain presents some difficulties. His outstanding complaint toward the end of his illness was intense vertigo, nausea and nystagmus produced by movements of the head. This had been interpreted as possibly due to a perilabyrinthitis. In view of the marked inflammation associated with inflammatory softening in the floor and lateral recesses of the fourth ventricle it seems that the patient probably had a so-called Bruns syndrome due to injury of the vestibular nuclei. Since the brain itself does not present changes adequate to cause death, it is likely that had block not supervened, the patient would have recovered.

Discussion. Although in the case described here, no attempts were made to identify the virus, the diagnosis of lymphocytic choriomeningitis was made on clinical grounds. Tuberculosis was ruled out by examination of spinal fluid by smear, culture, guinea pig inoculation and necropsy findings. Syphilis was excluded on basis of serologic tests upon blood and spinal fluid. The lack of any evidence of intraparenchymal disease of the central nervous system and the presence of an almost pure meningeal picture tended to exclude encephalitis. The high mononuclear content of the spinal fluid argued against non-paralytic poliomyelitis; further exclusion was made on necropsy findings, there being almost a complete absence of disease of the motor nuclei.

Further evidence that our case falls into the group called lymphocytic choriomeningitis is had in the resemblance of the necropsy findings to those reported in experimental monkeys by Lillie.[7] In his series of monkeys, the meninges showed slight to moderate focal to diffuse lymphocytic infiltration most marked in the sulci and fissures. The choroid plexuses showed at least a focal lymphocytic infiltration, often they were swollen and densely infiltrated with lymphocytes, serous exudates, macrophages and plasma cells. There was desquamation of the ependyma from the walls and plexuses in a number of animals. In a few animals subependymal edema and focal infiltration with lymphocytes were found. An explanation of

why this virus attacks the choroid plexus and ependyma cannot be made.

Contrasting the necropsy findings in our case with those reported by Viets and Warren,[10] differences are found insofar as Viets' and Warren's case showed evidence of encephalitis; in our case, the perivascular inflammatory reaction was sharply limited to the subependymal zones.

An interesting similarity between our case and that reported by Barker and Ford[3] is that in which a patient had an episode diagnosed as lymphocytic meningitis; the virus was identified by Rivers and Scott. Four months after the acute episode she developed evidences of severe arachnoiditis which caused obliteration of the spinal subarachnoid space. When Dandy explored the lower thoracic cord, a thick fibrous mass was found below the dura, completely obliterating the subarachnoid space in the region examined. A basal arachnoiditis was found in our patient which had caused a blockage of the cerebro-spinal fluid exit and gave rise to distention of the ventricular system resulting in respiratory failure.

Of course the absence of virus studies prevents absolute knowledge of the nature of the case; but the clinical picture, similarity of pathologic findings with those found in experimental monkeys and the death of two guinea pigs following inoculation with spinal fluid with no evidence of tuberculosis and at a time when there was no evidence of any virus infection among guinea pigs, made us believe that our case falls into the group labelled acute lymphocytic choriomeningitis. A case referred to by Lucchesi[8] was found to have evidence of poliomyelitis at necropsy.

As time goes on and more fatal cases are reported, it may be found that the disease has its specific fatal characteristics and in view of this, the term "benign" may no longer be appended. We had expected to find evidences of encephalitis at necropsy, but found none.

Summary. The clinical course, laboratory and necropsy findings of a patient believed to be suffering from lymphocytic choriomeningitis is presented. The chief changes in the brain (see pathological summary) were found in the ventricular walls, choroid plexuses and the basal meninges. No inclusion bodies were found. Death was due to respiratory failure secondary to increased intracranial pressure as the result of blockage of subarachnoid spaces at the base of the brain.

The authors wish to express their appreciation to Dr. B. J. Alpers of the department of Neuro-pathology for preparation of sections of the brain, and to Dr. J. S. Forrester of the Pepper Laboratory for use of data on the guinea pig inoculations with spinal fluid.

REFERENCES.

(1.) **Armstrong, C.,** and **Dickens, P. F.**: Pub. Health Rep., **50**, 831, 1935. (2.) **Armstrong, C.,** and **Wooley, J. G.**: J. Am. Med. Assn., **109**, 410, 1937. (3.) **Barker, L. F.,** and **Ford, F. R.**: Ibid., p. 785. (4.) **Dummer, C. M., Lyon, R. A.,** and **Steven-**

son, F. F.: Ibid., **108**, 633, 1937. (5.) **Findlay, G. M., Alcock, N. S.,** and **Stern, R. O.**: Lancet, **1**, 650, 1936. (6.) **Hughes, W.**: Brit. Med. J., **1**, 1063, 1937. (7.) **Lillie, R. D.**: Pub. Health Rep., **50**, 203, 1936. (8.) **Lucchesi, P. F.**: J. Am. Med. Assn., **108**, 1494, 1937. (9.) **Rivers, T. M.,** and **Scott, T. F. M.**: Science, **81**, 439, 1935. (10.) **Viets, H. R.,** and **Warren, S.**: J. Am. Med. Assn., **108**, 357, 1937 (11.) **Wallgren, A.**: Acta pædiat., **4**, 158, 1925.

TOXICOLOGY OF FLUORIDES.

By Alexander O. Gettler, Ph.D.,

PROFESSOR OF CHEMISTRY, WASHINGTON SQUARE COLLEGE; PROFESSOR OF TOXICOLOGY, MEDICAL SCHOOL OF NEW YORK UNIVERSITY; PATHOLOGICAL CHEMIST AND TOXICOLOGIST TO BELLEVUE HOSPITAL, NEW YORK CITY, N. Y.,

AND

Lester Ellerbrook, Ph.D.,

TOXICOLOGIST, CITY OF KINGSTON LABORATORIES, KINGSTON, N. Y.

(From the thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Washington Square College, New York University.)

1. **Methods for the Detection of Fluorine in Human Tissues.*** Many qualitative tests have been devised for the detection of fluorine. The outstanding ones are: the spectrographic test,[49] the Lanthanum fluoride precipitation,[46] the silico-molybdic acid test,[16,19] the bleaching of the zirconium-alizarin lake,[15] the zirconium oxychloride purpurin,[37] or the zirconium quinalizarin,[60] the zirconium salt of para-dimethyl amino-azo-phenyl arsenic acid spot test,[20] the etch test,[11] the precipitation of silicic acid from the volatilized silicon fluoride in the hanging drop,[41b] the sodium fluosilicate crystal test,[7] and the barium fluosilicate crystal test.[7] We have applied all these methods to the detection of fluorine in human tissues, and have found that most of them are not sensitive enough, since the fluorine content in the organs of a fatal poisoning is very often as little as 40 γ in 10 gm. of tissue. Although it has been stated[11] that by proper technique the etch test can be made quite sensitive, our investigations have shown that although it is sensitive enough for the detection of fluorine in the gastro-intestinal tract in cases of acute fluorine poisonings, because the fluorine content is relatively large, it is not sensitive enough for the detection of fluorine in blood and in the internal organs. Most of the above methods are not specific tests for fluorine: phosphates, sulphates, iron, and other substances, which may pass over with the fluorine in the form of a spray during the distillation of the fluorine, give the same reactions as does the fluorine.

2. **Technique Best Adapted for the Detection of Fluorine in Tissues.** In the toxicologic examination of a suspected case of poisoning by fluorine it is necessary to detect the fluorine, not only in the gastro-intestinal tract, but also in the internal organs. The amount of

* The term "fluorine" used throughout this paper designates fluoride ions originating from soluble fluorides, fluosilicates, or hydrofluoric acid.

fluorine present in the organs of a fatal fluorine poisoning is relatively small (quite often as little as 40 γ in 10 gm. of tissue).

Formerly, the etch test was applied to the ashed tissues. The method has the disadvantage that it is tedious and time-consuming completely to ash the tissues at the low temperature essential to prevent any volatilization of the alkali fluorides. Then again, since the test is not very sensitive, at least 100 gm. of tissue must be ashed, while 10 gm. or less are sufficient when our modified sodium fluosilicate test (described below) is used.

The primary difficulty in the detection of fluorine in so complex a medium as tissues and blood is the removal of the organic material. The ashing process is slow, and it may result in the loss of much of the fluorine if it is not carefully controlled. For this reason several other methods of preparing the material for analysis were studied in order to develop a quick convenient procedure. The method finally adopted follows:

Ten gm. of the minced tissue are placed in a 100-cc. beaker, an equal volume of water and 3 cc. of concentrated nitric acid are added, and the material is thoroughly mixed. After 3 to 5 minutes, 30 cc. of water are added with constant stirring. The material is then filtered, preferably by means of a Buchner funnel and suction. The filtrate containing the fluorine is transferred to a 100-cc. beaker, 0.5 to 1 gm. of solid lanthanum acetate, and enough solid ammonium acetate is added until the solution is definitely alkaline to methyl orange, whereupon a gelatinous precipitate of lanthanum salts appears. The mixture is boiled for a few minutes in order to coagulate the precipitate. After the precipitate has been allowed to settle, the supernatant liquid is filtered through a small Gooch crucible by aid of suction. The precipitate is finally also transferred to the Gooch by means of a stream of water. Since this gelatinous precipitate filters slowly, the following procedure may be resorted to: After the supernatant fluid has been decanted into the Gooch crucible, the gelatinous precipitate remaining in the beaker is dried, and heated to approximately 150° C. for a few minutes. The sandy residue is boiled up with a few cc. of a dilute acetic acid ammonium acetate mixture, and then filtered through the same Gooch crucible, and washed with a 1% lanthanum acetate solution. The asbestos mat holding the precipitate is transferred to a 5-cc. porcelain crucible, and dried on a hot plate. When cool, a little powdered glass is mixed with the dry material, a few drops of sulphuric acid are added, and the crucible is immediately covered with a microscope slide from the under surface of which is suspended a small drop of 5% NaCl solution. The crucible is placed on a small metal block maintained at a temperature of approximately 150° C. and a small beaker of ice-cold water is placed on the slide in order to retard the evaporation of the suspended drop. If relatively large amounts of fluorine are present the heating can be omitted. After 3 to 5 minutes the slide is removed, and examined under the microscope (high magnification) for 6-pointed stars or slightly pink hexagonal crystals of sodium fluosilicate.

It is not absolutely necessary to use 10 gm. of tissue in this test, for a 5-gm. sample from a case of poisoning by fluorine always yields definite crystals of sodium fluosilicate. One can easily detect as little as 10 γ of fluorine in 5 gm. of tissue by this procedure.

When this test was applied to 50-gm. portions of normal tissue, or normal blood, 3 or 4 crystals of sodium fluosilicate in the entire

field were observed, due to the normal fluorine content. The results on 20-gm. portions of normal tissues and blood were always negative. A positive result, therefore, when 10 gm. or less of tissues are used, always indicates the presence of abnormal amounts of fluorine.

Stomach contents and urines in acute fluorine poisonings contain much larger amounts of fluorine than do the tissues. The method can, therefore, be simplified by using smaller samples and omitting the nitric acid and lanthanum treatment. One gm., or even less, of the material is placed in a 5-cc. crucible. It is made slightly alkaline with sodium hydroxide solution, and dried on a hot plate. A little powdered glass is mixed with the solid material, and enough sulphuric acid is added just to cover the mixture. The crucible is immediately covered with a microscope slide from which is suspended a small drop of 5% NaCl solution. From here on the procedure is the same as described above.

In acute fluorine poisonings, positive results have been obtained in this manner from as little as 1 drop of stomach contents or urine. If large amounts of stomach contents or urine are used, the method given for tissues must be used because of the excessive amount of organic material present.

The test can be used for corroborating the clinical diagnosis of fluorine poisoning, for the procedure is short and simple, and the sensitivity is such that fluorine can be detected in 5 cc. of blood, or 1 cc. of urine in such cases. The test gives a negative result when applied to 5 cc. of blood, or 1 cc. of urine taken from normal individuals.

3. **Methods for the Quantitative Determination of Fluorine.** All the available quantitative methods for fluorine were studied as to their applicability for the determination of small quantities of fluorine as found in human organs. The gravimetric methods consist in weighing the fluorine as calcium fluoride;[8,52,69] as lanthanum fluoride;[46] as potassium fluosilicate;[12] as lead chlorofluoride;[64] as triphenyl tin fluoride;[1] as silicon tetrafluoride after collecting the latter in an absorption tube; by driving off all of the fluoride as silicon tetrafluoride, and determining the loss in weight of the sample;[76] by determining the loss in weight of a glass funnel etched by hydrogen fluoride.[45] In the volumetric procedures, the fluorine in in the form of silicon tetrafluoride is distilled into water, and the fluosilicic acid produced is titrated with standard potassium hydroxide solution;[48] or the fluosilicic acid is treated with potassium chloride, and the hydrochloric acid produced is titrated with standard sodium hydroxide solution;[50] a solution of the neutral fluoride salt is titrated with standard ferric chloride using potassium thiocyanate as indicator;[28,70] the neutral fluoride salt is treated with an excess but known quantity of ferric chloride solution, and the excess is determined by adding potassium iodide, and titrating the liberated iodine with standard sodium thiosulphate;[18,21b] titrating the fluorine with standard cerium nitrate:[6,32,56] or with standard zirconium

oxychloride solution, using purpurin as indicator;[37] titrating the fluorine with standard thorium nitrate solution, using zirconium alizarin lake[75] or zirconium quinalizarin[60] or thorium alizarin lake[3] as indicators. The colorimetric methods are based on the bleaching action of fluorine ions upon colored substances such as peroxytitanic acid;[66,72] ferric thiocyanate;[22] ferric acetyl acetone;[5,74] and various zirconium lakes.[17,37,53,60]

Our studies as to the application of the various gravimetric, volumetric and colorimetric procedures to the quantitative determination of small amounts of fluorine, in human tissues, has revealed that the thorium nitrate-alizarin volumetric method[3] is best adapted from a standpoint of accuracy, sensitivity and simplicity of technique.

4. **Technique for the Quantitative Determination of Fluorine in Tissues.** A 50-gm. portion of finely ground tissue in a porcelain or silica dish is thoroughly mixed with 2% of its weight of Na_2CO_3 (20 cc. of 5% solution). After drying on a steam bath, the material is placed into an electric muffle and kept at dull red heat until completely charred, and no more volatile organic vapors are seen coming off. The material must not be allowed to glow. The charred mass is then pulverized, replaced into muffle and ashing continued at dull red heat for 2 to 3 hours. The black ash is then leached, by boiling with 100 to 200 cc. of water. The supernatant liquid is filtered into a 400-cc. beaker. A few cc. of 50% perchloric acid and a little water is added to the residue in order to dissolve any CaF_2 if present. Filtration is completed and the black residue on the filter paper is washed free of any perchloric acid, adding the washings to the main solution in the beaker. To the residue and filter paper is added 0.25 gm. Na_2CO_3 (in solution), well mixed, and dried on steam bath. The ashing of the residue is now completed in muffle at dull red heat, until a grayish-white ash is obtained. The ash is dissolved by adding 5 cc. of 10% perchloric acid and then quantitatively washed into the main solution in the 400-cc. beaker. The mixture thus obtained is made alkaline, to phenolphthalein, with NaOH solution, and then evaporated to 20-cc. volume. It is now quantitatively transferred to a 150-cc. distillation flask, several small glass beads are added, 25 cc. of 60% perchloric acid are slowly introduced and water added to make the total volume between 80 and 100 cc., so that the solution may have a boiling point of about 110° C. Potassium carbonate is slowly added with constant mixing, until a small crystalline precipitate of potassium perchlorate forms. These crystals serve as nuclei for the formation of bubbles of steam which prevent bumping during the distillation. A small amount of freshly prepared Ag_2O is added to prevent the distillation of any chloride (as HCl). The distilling flask is then set up for distillation, resting in a $2\frac{1}{4}$-inch diameter opening of an asbestos mat. The neck of the flask is fitted with a 2-hole rubber stopper, through which passes a thermometer and a capillary tube, both of which extend into the liquid in the flask. A dropping funnel is connected to the upper part of the capillary tube so that water can be added, gradually, during the distillation. The side arm of the distilling flask is connected with a long water-cooled condenser, and adaptor, the latter dipping into 2 cc. of 1% NaOH solution in a 500-cc. receiving flask. The distillation is then carried on until the boiling point rises to 140° C. It is kept between 135° and 140° C. through the entire distillation, by gradually introducing water through the capillary tube; 250 cc. of the distillate are collected, because our experiments on this point indicated this amount of distillate is required for complete recovery of the fluorine.

The distillate containing the fluorine, while still alkaline, is evaporated to a volume of about 5 cc., then quantitatively transferred to a 30-cc. test tube, and the volume made up to 10 cc. with water. For very small quantities of fluorine (order of 10 γ) the volume is limited to 3 cc. To this solution are now added 10 cc. of ethyl alcohol, 0.03 cc. of a 0.05% aqueous solution of sodium alizarin sulphonate, dilute (1 to 50) HCl dropwise until the transition color (yellow) of the indicator appears, and 1 cc. of a sodium hydroxide-chloracetic acid buffer (1 gm. NaOH + 4.72 gm. of chloracetic acid in 100 cc. of solution).*

A blank reference standard is prepared by placing 20 cc. of 50% alcohol into a similar test tube, and adding the same quantity of the alizarin indicator and buffer as above. Standard thorium nitrate solution (0.001 N) is then added from a micro-burette until a faint pink color appears. This pink color is the reference standard.

The fluorine content of the solution prepared above is now titrated by slowly adding 0.001 N or 0.01 N (depending on the amount of fluorine expected to be present) thorium nitrate solution, from a micro-burette, until a faint orange color develops. The titration is then completed by adding a fraction of a drop of the standard solution, per minute, until the solution assumes the same pink color of the blank reference standard previously prepared. The volume of standard thorium nitrate solution required in the preparation of the reference standard is then deducted from the actual titration figure.

Calculation. Since 1 cc. of 0.01 N thorium nitrate solution is equivalent to 190 γ of fluorine, and 1 cc. of 0.001 N to 19 γ, the number of cc. of standard thorium nitrate used, multiplied by the fluorine equivalent, gives the fluorine content of the 50-gm. portion of tissues used for analysis. Somewhat more accurate results are obtained if one determines the fluorine content from the titration figures obtained by titrating a series of known fluorine solutions.

5. **The Fluorine Content of Normal Human Tissues.** Since fluorine is so widely distributed in Nature, it is not surprising to find it present in normal human tissues. The values reported in the literature vary widely, due no doubt in most cases to the inaccurate methods of analysis employed. Some of the values are given in Table 1.

The presence of much larger amounts of fluorine in the bones and teeth than in soft tissue has been definitely established, but the amounts reported in the literature vary as much as from 0.0%[21] to 1.55%.[77] It has also been shown that fluorine accumulates in the bones and teeth when small amounts of fluorine are present in the daily diet.[10,14,35,51,58,62]

In order definitely to establish the true fluorine content of normal human soft tissues, bones, teeth, blood and urine, a series of analyses were conducted. Both the calcium hydroxide and sodium carbonate methods of ashing were employed. Controls were included to which definitely known amounts of fluorine had been added. The results are recorded in Table 2.

That the material titrated was actually fluorine was confirmed in the case of each tissue as follows: After the titration had been completed, the precipitate of thorium fluoride was centrifuged down.

* Suggested by Hoskins and Ferris[31] for controlling the pH during the titration.

The supernatant liquid was poured off, and the precipitate was washed into a 5-cc. crucible. A drop of dilute sodium hydroxide was added and the solution was evaporated to dryness. The presence of fluorine in the precipitate was demonstrated by the typical sodium fluosilicate crystal test.

TABLE 1.—FLUORINE CONTENT OF NORMAL HUMAN TISSUES REPORTED BY VARIOUS INVESTIGATORS. (EXPRESSED IN MG. PER 100 GM. TISSUE.)

Blood	0.35	Zdareck.
	0.46	Gautier and Clausmann.
	0.05–0.10	Goldemberg and Schreiber.
	None	Stuber and Lang.
	3.97 (in hemophilia)	Stuber and Lang.
	0.02	Brandes
Brain	0.06–0.07	Zdareck.
	0.71 (adult)	Gautier and Clausmann.
	0.18 (newborn)	Gautier and Clausmann.
Liver	0.21–0.31	Zdareck.
	0.64 (adult)	Gautier and Clausmann.
	0.37 (newborn)	Gautier and Clausmann.
Kidneys	0.45–0.51	Zdareck.
	0.26	Gautier and Clausmann.
Heart	0.16	Zdareck.
Lungs	0.09–0.17	Zdareck.
	0.44	Gautier and Clausmann.
Spleen	0.27–0.62	Zdareck.
Muscles	0.16	Gautier and Clausmann.
Thyroid	0.54	Gautier and Clausmann.
Urine	0.018	Gautier and Clausmann.
	0.03–0.17	Goldemberg and Schreiber.
Feces	0.42	Gautier and Clausmann.
	0.04–0.07	Goldemberg and Schreiber.
Bile	0.04–0.07	Goldemberg and Schreiber.
Milk	0.048	Gautier and Clausmann.
	0.04–0.07	Goldemberg and Schreiber.
Femur	0.29	Zdareck.
Humerus	0.35–0.45	Zdareck.

The data in Table 2 reveal the following interesting points: (*a*) Very small amounts of fluorine are found in normal human tissues (0.00002 to 0.00008%) (20 to 80 γ in 100 gm.). (*b*) There seems to be no appreciable accumulation of fluorine in any one of the vital organs. (*c*) The values reported by previous investigators range from 0.000000 to 0.00397%[67] (0.000 to 4000 γ in 100 gm.). The present investigation disproves the entire absence of fluorine, as well as the high values and wide variations which have been reported. (*d*) The fluorine content of teeth and bones is of the order of 0.01 to 0.03%. The actual value undoubtedly varies with the amount of fluorine in the daily diet. (*e*) The fluorine content of the urine is of the same order of magnitude as that of the tissues. It is to be expected, however, that it would vary with the amount of fluorine in the diet. (*f*) The results of the control analyses, to which definitely known quantities of fluorine were added, confirm the accuracy of the method.

TABLE 2.—FLUORINE CONTENT OF NORMAL HUMAN TISSUES.

Tissue used for analysis, gm.	Ashing method.	Fluorine added, mg.	Fluorine found, mg.	Fluorine in wet tissue, %.
Liver (1) . . . 50	$Ca(OH)_2$	..	0.030	0.000060
50	Na_2CO_3	..	0.035	0.000070
50	$Ca(OH)_2$	..	0.030	0.000060
Liver (2) . . . 50	Na_2CO_3	..	0.020	0.000040
50	Na_2CO_3	0.500	0.520	0.000040
50	$Ca(OH)_2$	0.100	0.118	0.000036
Liver (3) . . . 100	$Ca(OH)_2$	..	0.065	0.000065
100	$Ca(OH)_2$	..	0.055	0.000055
Kidney (1) . . 50	$Ca(OH)_2$	..	0.039	0.000078
50	$Ca(OH)_2$	..	0.039	0.000078
50	$Ca(OH)_2$	0.500	0.535	0.000070
Kidney (2) . . 50	$Ca(OH)_2$	..	0.028	0.000056
50	Na_2CO_3	..	0.030	0.000060
50	Na_2CO_3	0.050	0.080	0.000060
Blood (1) . . . 100	$Ca(OH)_2$	..	0.020	0.000020
Blood (2) . . . 50	Na_2CO_3	..	0.010	0.000020
Blood (3) . . . 50	Na_2CO_3	..	0.021	0.000042
Blood (4) . . . 100	$Ca(OH)_2$	..	0.064	0.000064
Brain (1) . . . 50	Na_2CO_3	..	0.028	0.000056
Brain (2) . . . 50	Na_2CO_3	0.050	0.084	0.000068
Brain (3) . . . 50	$Ca(OH)_2$	0.500	0.525	0.000050
Brain (4) . . . 50	$Ca(OH)_2$	0.500	0.520	0.000040
Lung (1) . . . 50	Na_2CO_3	..	0.008	0.000016
Lung (2) . . . 50	Na_2CO_3	..	0.012	0.000024
Lung (3) . . . 100	Na_2CO_3	..	0.042	0.000042
Heart (1) . . 50	Na_2CO_3	..	0.030	0.000060
Heart (2) . . . 100	$Ca(OH)_2$	..	0.044	0.000044
Spleen (1) . . 50	Na_2CO_3	..	0.015	0.000030
Spleen (2) . . 100	$Ca(OH)_2$	..	0.025	0.000025
Femur (1) . . 7.80	Direct	..	0.930	0.0119
Femur (2) . . 4.80	Direct	..	0.535	0.0112
Femur (3) . . 3.21	Direct	..	0.454	0.0141
Teeth (1) . . . 2.00	Direct	..	0.620	0.0310
Teeth (2) . . . 1.53	Direct	..	0.421	0.0275
Enamel . . . 0.36	Direct	..	0.070	0.0190
Urine (1) . . . 200	$Ca(OH)_2$	..	0.080	0.000040
Urine (2) . . . 250	Na_2CO_3	..	0.130	0.000052
Urine (3) . . . 250	Na_2CO_3	..	0.140	0.000056
Urine (4) . . . 250	Na_2CO_3	..	0.120	0.000048

6. **The Distribution of Fluorine in the Body Tissues in Fatal Cases of Fluorine Poisoning.** The fluorine content of some of the tissues in fatal cases of fluorine poisoning have been reported. Luhrig[41] found 0.00103% in the brain and 0.00214% in the liver of a fatal case of sodium fluosilicate poisoning. In another of his cases the liver contained 0.0015% fluorine.[41b]

Sharkey and Simpson[57] found 0.0837% in the liver and 0.0543% in kidney tissue. McNally[43] reports the following percentages of fluorine in 3 poisoning cases: Liver, 0.0025 to 0.1142; kidneys, trace to 0.0591; spleen, 0.1065; blood, 0.0654; lungs, 0.0706; heart, 0.0880; pancreas, none.

In Table 3 we have charted our findings on the fluorine content of several organs of 5 fatal acute cases of sodium fluoride poisoning. Cases 2, 4 and 5 were brought to the hospital and died in less than 24 hours. Cases 1 and 3 were found dead.

It is interesting to note that in 2 of our fatal cases the fluorine content of the tissues at the time of death was less than some of the values which have been reported by several investigators for normal tissues. On the other hand, the fluorine content of the organs of these same 2 fatal cases is twice as high as the highest normal fluorine content as determined by our technique. This seems further to corroborate the fact that the normal fluorine content of organs reported in the literature is much too high.

TABLE 3.—FLUORINE CONTENT OF HUMAN TISSUES IN FATAL CASES OF POISONING (VALUES REPORTED IN PER CENT).

Tissue.	Case 1.	Case 2.	Case 3.	Case 4.	Case 5.
Blood	0.00155	0.00121	0.00108	0.00042	0.00035
Spleen	...	0.00118			
Kidney	...	0.00116	0.00107	0.00046	
Brain	...	...	...	0.00034	0.00016
Heart	...	0.00106			
Lung	0.00156	0.00124			
Liver	0.00150	0.00122	0.00106	0.00044	0.00044

Since all of the organs and tissues in our fatal cases were not submitted for analysis and since the fluorine distribution in all tissues was desired, a series of experiments was carried out in which dogs were used as subjects.

Sodium fluoride was administered to each of 3 dogs, Dogs 1, 2, 3, and sodium fluosilicate to 2 others, Dogs 4 and 5, either in food or by stomach tube. The symptoms observed were persistent vomiting, salivation and lachrymation, stupor and weakness, muscular twitchings, diarrhea, acceleration and deepening paralysis, terminal clonic convulsions, and early rigor. In all cases the heart beat continued for several minutes after cessation and respiration. The heart's blood, drawn immediately after death, was of a dark red color; clotting occurred within a few seconds but with little retraction of the clot and separation of serum.

The results of the analyses of various body tissues of these experimental animals are recorded in Table 4.

TABLE 4.—FLUORINE CONTENT (%) OF TISSUES IN EXPERIMENTAL CASES OF POISONING BY FLUORIDE AND FLUOSILICATE.

Tissue.	Case 1.	Case 2.	Case 3.	Case 4.	Case 5.
Pancreas	0.00036	0.00058	0.00049		
Spleen	0.00039	0.00060	0.00052	0.00148	
Blood	0.00045	...	0.00103		
Kidney	0.00031	0.00064	0.00066	0.00150	0.00123
Brain	0.00014	0.00018	0.00018	0.00048	0.00036
Heart	0.00021	0.00054	0.00051	0.00111	0.00098
Lung	0.00035	0.00063	0.00076	0.00149	0.00122
Liver	0.00036	0.00066	0.00076	0.00150	0.00122
Muscle	0.00020	0.00042	0.00040	0.00102	0.00081
Urine	...	...	0.0062		
Femur	0.0393	0.0160	0.0401	0.0281	0.0326
Teeth	0.0300	0.0163	0.0234	0.0234	0.0261
Time of life (hrs.)	26	7	9	8	8
Weight (kg.)	9.1	11.2	9.2	9.5	9.8

The data of Tables 3 and 4 bring out the following points of interest: (*a*) In acute deaths, the fluorine content of the internal organs in humans is within the same range as in dogs, namely, 0.00014 to 0.0016% (0.14 to 1.6 mg. in 100 gm.). (*b*) The amount of fluorine found in the tissues in fatal cases of poisoning by sodium fluosilicate was approximately the same as that found when death was caused by sodium fluoride. (*c*) The fluorine content of the brain in every case was somewhat smaller than that of any other organ. (*d*) The fluorine content of the muscles was somewhat lower than the organs but a little higher than the brain. (*e*) The fluorine content of the bones and teeth no doubt has also increased but the increase cannot be calculated because of the wide variation of their normal fluorine content. (*f*) The fluorine content of the urine may vary a great deal: in 1 case it was approximately 10 times that of the tissues.

In order to determine whether there is an accumulation of fluorine in the various organs during chronic fluorine poisoning, the following experiment was conducted. Each of the 2 dogs (6 and 7) was given 18 mg. and 32 mg. of sodium fluoride per kilo of body weight respectively, every day for a period of 2 weeks. The animals were destroyed 48 hours after the ingestion of the last dose, and the tissues were subjected to analysis. Some of the tissues of 2 normal dogs (8 and 9) were used in control analyses. The results of these analyses are recorded in Table 5.

TABLE 5.—FLUORINE CONTENT (%) OF TISSUES IN NORMAL AND CHRONIC FLUORINE CASES.

Tissue.	Chronic.		Normal.	
	Dog 6.	Dog 7.	Dog 8.	Dog 9.
Pancreas	0.000020	0.000042	...	0.000027
Spleen	0.000021	...	...	0.000041
Blood	0.000023	0.000033	0.000025	0.000028
Kidney	0.000040	...	...	0.000042
Brain	0.000031	0.000035	0.000031	
Heart	0.000050	0.000041	0.000044	
Lung	0.000022	...	0.000040	
Liver	0.000031	0.000043	0.000026	0.000048
Muscle	0.000033	...	0.000030	
Femur	0.0200	0.0182	0.0250	0.0269
Teeth	0.0313	0.0142	...	0.0203

The results of this experiment indicate that the continued ingestion of small amounts (18 and 32 mg.) of NaF per kilo per day for a period of 2 weeks does not result in any accumulation of fluorine in the internal organs. The reason why the organism can tolerate doses as large as these (which in the average man would amount to about 1 gm. of fluorine per day) is twofold: (*a*) The absorption of fluorine from the intestines is relatively slow, due to the conversion of some of the soluble fluoride into difficultly soluble calcium fluoride; and (*b*) fluorine in the circulation is very rapidly excreted by way of kidneys.

7. **The Minimum Lethal Dose of Fluorine.** Various values for the lethal dose of fluorine have been reported. In a case reported by McNally,[43] the dose taken was 4.5 to 5 gm. of 90% NaF. Luhrig[41a] reports a fatal case of poisoning from 4 gm. (½ teaspoonful) Na_2SiF_6. Wieland and Kurtzahn[73] estimate the fatal dose of Na_2SiF_6 as 0.045 to 0.09 gm. per kilo. Baldwin[5] cites 3 cases that recovered from 5, 6 and 9 gm. of NaF each, taken in wheat cakes. He himself took 0.03 gm. NaF with no ill-effects, but 0.25 gm. gave him nausea which lasted about 24 hours. Marcovitch[44] estimates the lowest lethal dose of NaF to be 30 gm. (approximately 0.4 gm. per kilo) and that of Na_2SiF_6 as 7.2 gm. (0.1 gm. per kilo). Starkenstein, Rost and Pohl[65] state that the fatal dose of NaF is 0.1 to 0.2 gm. per kilo while that of Na_2SiF_6 is 0.04 to 4.14 gm. per kilo. Fullerton[32] reports a case in which death occurred after the ingestion of approximately 4 gm. of NaF, and Gellerstedt[24] estimates that as little as 0.7 gm. of Na_2SiF_6 has caused death.

The reason why such wide variations as to the lethal dose exists may be (*a*) the amount of fluorine actually taken by the subject is only a guess; (*b*) some of the ingested fluorine is lost in the vomitus; (*c*) the fluorine remaining in the gastro-intestinal tract (unabsorbed) has no bearing on the cause of death; that part of the poison which has been absorbed into the circulation and tissues is solely responsible for the death of the individual; (*d*) fluorine is very rapidly excreted in the urine.

More accurate information on the minimum lethal dose exists when the experimental data obtained by animal experimentation are considered, although here also there is some disagreement. Heidenhain[29] found that 0.05 to 0.1 gm. of NaF per kilo intravenously to a dog was fatal. Sollmann[61] states that 8-mg. daily doses of NaF per kilo of body weight to rats produced no deleterious effect within 9 weeks, but 15 to 150 mg. per kilo resulted in progressive impairment of both growth and food consumption, with little mortality and no histologic lesions. Marcovitch[44] reports that 6.06 mg. of fluorine (as Na_2SiF_6) per kilo introduced into a rabbit intravenously was fatal in 2 minutes. Leake[39] states that the minimum lethal dose for rabbits intravenously is 0.0875 gm. NaF per kilo. Muehlberger[47] found 20.8 mg. of fluorine (as NaF) to be a lethal dose for rats when administered subcutaneously. Goldemberg[25] found the minimum lethal dose by the intraperitoneal route to be 12.7 to 15.8 mg. of fluorine (as NaF) per kilo of body weight.

Muehlberger[47] determined the relative toxicities of NaF, Na_2SiF_6, and $BaSiF_6$ on rats and rabbits. His data seem to confirm the statement of Wieland and Kurtzahn[73] that fluorides and fluosilicates are toxic in proportion to their fluorine content. Weight for weight the latter is 3 to 4 times more toxic than the former.

This is what might have been expected, for Na_2SiF_6 is partly converted to NaF and SiF_4 in pure water solution, while in alkaline solution the reaction $Na_2SiF_6 + 4NaOH = 6NaF + Si(OH)_4$ takes

place rapidly. Since the intestine is an alkaline medium, it is only natural to suppose that when fluosilicates are ingested this change occurs almost completely before absorption occurs.

In order to test the validity of this supposition, attempts were made to establish the presence of fluosilicate ions in the intestinal tract of the dogs to which this substance had been administered. To a few drops of intestinal washings on a celluloid microscope slide was added a drop of sodium chloride solution. No crystals of Na_2SiF_6 were observed under the microscope. Similar attempts to obtain the crystals of $BaSiF_6$ were also unsuccessful. In order to rule out the possibility that all of the fluosilicate had already been absorbed, the presence of fluorine in the intestinal washings was demonstrated by the application of the hanging drop test with the production of crystals of Na_2SiF_6.

It was our intention to calculate the minimum lethal absorbed dose of fluorine (exclusive of the gastro-intestinal tract) from the analysis of the various organs and tissues. This was impossible, however, because of the fact that fluorine accumulates in the bones, teeth, fat, skin, hair and nails, during life, from the daily diet, and since the normal fluorine content of these tissues varies over a wide range and is not known for any specific case, the amount of absorbed fluorine in these tissues originating from the dose of fluorine cannot be estimated. We have therefore simply calculated the minimum absorbed lethal dose in the body exclusive of bones, teeth, fat, skin, hair and nails.

TABLE 6.—LETHAL FLUORINE CONTENT OF INTERNAL ORGANS.

	Weight (gm.) of organ in body of 140 lbs.	Fluorine % of wet tissue smallest found.		Smallest amount of fluorine (mg.) in entire organ.
Brain	1,250	human	0.00016	1.75
		dog	0.00014	
Liver	1,500	human	0.00040	5.40
		dog	0 00036	
Kidneys	300	human	0.00046	1.14
		dog	0.00031	
Heart	300	dog	0.00021	0.63
Lungs	800	dog	0.00035	2.80
Spleen	150	dog	0.00039	0.58
Pancreas	100	dog	0.00036	0.36
Muscles	30,000	dog	0.00026	78.00
Blood	4,000	human	0.00035	14.00
		dog	0.00035	
Total wt. analyzed organs.	38,400	.....		104.66
Bones, fat, G.I. tract, skin, hair, etc.	24,600	Not included		Not included
Total body wt.	63,000			

The data of Table 6 bring out the following points: (*a*) The smallest lethal dose of fluorine absorbed into the internal organs in our cases was 104.66 mg. (calculated to a body weighing 63 kilos = about 140 pounds). For bodies of different weight, the fluorine content of the organs can be calculated by using the values in the per cent column; (*b*) multiplying the combined fluorine content of

the liver and the brain by 15, one obtains a fair estimate of the total amount of fluorine present in all of the internal organs.

8. **Symptoms of Fluorine Poisoning.** *Acute poisoning*: from NaF or Na_2SiF_6 *per os:* (1) Gastro-intestinal disturbances: nausea, vomiting, purging, convulsive pains in abdomen. (2) Salivation and lachrymation. (3) Pulmonary disturbances: acceleration and deepening of respiration followed by dyspnea. (4) Cardiac insufficiency and cardiac weakness. (5) Motor and sensory disturbances: tremors and convulsions which may involve only a part or the whole body, and may assume an epileptic character. These symptoms, according to Tappeiner[68] are not of a reflex nature, and are not dependent upon disturbances of the respiration and circulation, but originate in the spinal cord and higher centers. Paralysis of the vasomotor center; patient may be unable to open his mouth; loss of speech. Disorder of sensibility of fingers. Convulsive pains in legs, and uncertainty of gait. (6) Signs of renal irritation, albumin in urine and edema. (7) Terminal stage: general exhaustion, loss of consciousness, respiratory paralysis. Heart continues to beat a minute or two after cessation of respiration. Death in several hours.

Acute poisoning with a solution of hydrofluoric acid *per os:* There is severe pain in abdomen almost immediately, with vomiting and purging. Many of the symptoms listed under sodium fluoride poisoning are also in evidence. Death results from cardiac paralysis and occurs in 1 to 6 hours.

Acute poisoning by inhaling hydrogen fluoride gas: The inhalation of HF is very deleterious. The Belgian chemist, Louyet, died from the inhalation of the gas, while experimenting with it. Severe laryngitis and bronchitis result. The lesions are ulcerations of the conjunctiva, nose, mouth and gums. Severe pain in throat and stomach, with vomiting and collapse.

Action of a hydrofluoric acid solution upon the skin: The solution of this acid in contact with the skin produces severe and very painful burns and blisters, which heal very slowly.

Chronic Poisoning. From small daily doses of sodium fluoride: Sollmann[61] states that 8-mg. daily doses of NaF per kilo of body weight in rats, for 9 weeks, produced no deleterious effects on growth or food consumption. Daily doses of 15 to 150 mg. of NaF per kilo body weight resulted in progressive impairment of growth and food consumption, little mortality, with no histologic changes. In our experiments, 18 and 32 mg. daily doses per kilo for 2 weeks produced no symptoms except that the dogs lost their appetite. After somewhat larger amounts, 50 to 100 mg. per kilo for several months, the animal walked with difficulty, his back sagged, hair fell out and teeth chipped off.

9. **Postmortem Findings.** Due to swallowing of alkali fluorides: Premature rigor mortis. Severe hemorrhagic gastritis, swelling and corrosion of stomach mucosa with dark red discoloration, and extravasations of blood. The dark crimson discoloration of the rugæ is

very pronounced. Veins are filled with viscous dark red blood. Kidneys give evidence of nephrosis.

Due to swallowing a solution of hydrofluoric acid: The buccal mucous membrane is white; the tongue, gums, epiglottis and esophagus are denuded of epithelium; the mucous membrane of the stomach is softened and corroded, very dark, almost black discoloration of the rugæ and intensely red and ecchymosed in the depressions.

10. **Treatment.** For alkali fluorides and fluosilicates taken internally: (1) Abundant stomach wash with lime water, milk, or an aqueous suspension of finely divided medicinal charcoal; (2) administer castor oil as a purgative; (3) give plenty of fluids in order to flush the system. For burns of the skin due to hydrofluoric acid: Quick action is essential. Thoroughly wash the affected skin with a 5% solution of soda-lime. Avoid rubbing the skin. Then bathe the affected portions in 1% soda-lime solution for about ½ hour, and apply some butesin picrate salve.

Summary. 1. Various qualitative tests and quantitative determinations for fluorine are discussed from the standpoint of their applicability to tissue analysis.

2. The technique, which in our hands gave the best result, for the detection and quantitative determination of fluorine, is described.

3. The normal fluorine content of the various human organs and tissues has been determined.

4. The distribution of the fluorine into the various organs and body tissues of fatal fluoride cases has been determined.

5. The minimum lethal absorbed dose of fluoride has been estimated.

6. The symptoms of fluorine poisoning are described.

7. Postmortem findings in fatal fluoride cases are described.

8. The treatment for fluoride poisoning is outlined.

REFERENCES.

(1.) **Allen, N.**, and **Furman, N. H.**: J. Am. Chem. Soc., **54**, 4625, 1932. (2.) **Amberg, S.**, and **Loevenhart, A. A.**: J. Biol. Chem., **4**, 149, 1908. (3.) **Armstrong, W. D.**: J. Am. Chem. Soc., **55**, 1741, 1933; Indust. and Engr. Chem., Anal. ed., **5**, 315, 1933; **8**, 384, 1936. (4.) **Arthus, M.**, and **Gavelle, J.**: Compt. rend. Soc. d. biol., **55**, 1481, 1903. (5.) **Baldwin, H. B.**: J. Am. Chem. Soc., **21**, 517, 1889. (6.) **Batchelder, G.**, and **Meloche, V. W.**: Ibid., **53**, 2131, 1931. (7.) **Behrens, H.**, and **Kley, P. D. C.**: Mikrochemishe Analyse, 4th ed., Leipzig, Leopold Voss, **1**, 177, 1921. (8.) **Berzelius, J. J.**: J. Chem. u. Physik, **16**, 426, 1816. (9.) **Brandes, W.**: Ztschr. f. klin. Med., **119**, 504, 1932. (10.) **Brandl, J.**, and **Tappeiner, H.**: Ztschr. f. Biol., **28**, 518, 1891. (11.) **Brunig, A.**, and **Quest, H.**: Ztschr. angew. Chem., **44**, 656, 1931. (12.) **Carnot, A.**: Ztschr. anal. Chem., **35**, 580, 1896. (13.) **Churchill, H. V.**: Indust. and Engr. Chem., **23**, 996, 1931. (14.) **Cristiani, H.**: Ann. d. hyg., **8**, 309, 1930. (15.) **de Boer, J. H.**: Chem. Weekblad, **21**, 404, 1924. (16.) **Eegriwe, E.**: Ztschr. anal. Chem., **65**, 184, 1924. (17.) **Elvove, E.**: Pub. Health Rep., **48**, 1219, 1933. (18.) **Fairchild, J. G.**: J. Washington Acad. Sci., **20**, 141, 1930. (19.) **Feigl, F.**, and **Krumholz, P.**: Mikrochemie, **5**, 83, 1929. (20.) **Feigl, F.**, and **Rajmann, E.**: Ibid., **12**, 133, 1932. (21.) **Foster, M. D.**: (*a*) J. Am. Chem. Soc., **54**, 4464, 1932; (*b*) Indust. and Engr. Chem., Anal. ed., **5**, 234, 1933. (22.) **Fullerton, W. W.**: New England J. Med., **203**, 423, 1930. (23.) **Gautier, A.**, and **Clausmann, P.**: (*a*) Compt. rend. Soc. d. biol., **154**, 1670, 1912; (*b*) Ibid., **157**, 94, 1913. (24.) **Gellerstedt, M.**: Deutsch. Ztschr. f. ges. gericht. Med., **19**, 475, 1932. (25.) **Goldemberg, L.**: J. physiol. et de pathol. générale, **28**, 556, 1930; Chem. Abstr., **24**, 5871,

1930. (26.) **Goldemberg, L.**, and **Schraiber, J.**: Rev. Soc. argent. biol., **11**, 43, 1935; Chem. Abstr., **29**, 7451, 1935. (27.) **Gottdenker, F.**, and **Rothberger, C.**: Arch. f. exp. Path. u. Pharm., **179**, 38, 1935. (28.) **Greef, A.**: Ber. d. deutsch. chem. Gesellsch., **46**, 2511, 1913. (29.) **Heidenhain, H.**: Pflüger's Arch. f. Physiol., **62**, 197, 1922. (30.) **Hoff, F.**, and **May, F.**: Ztschr. f. klin. Med., **112**, 558, 1930. (31.) **Hoskins, W. M.**, and **Ferris, C. A.**: Indust. and Engr. Chem., Anal. ed., **8**, 6, 1936. (32.) **Hubbard, D. M.**, and **Henne, A. L.**: J. Am. Chem. Soc., **56**, 1078, 1934. (33.) **Huddleston, L. J.**, and **Bassett, H.**: J. Chem. Soc., **119**, 403, 1921. (34.) **Jodlbauer, A.**: Arch. f. exp. Path. u. Pharm., **164**, 464, 1932. (35.) **Kick, C. H.**, **Bethke, R. M.**, and **Edgington, B. H.**: J. Agr. Res., **46**, 1023, 1933. (36.) **Kobert, R.**: Lehrbuch der Intoxikationen, Stuttgart, Enke, p. 200, 1906. (37.) **Kolthoff, I. M.**, and **Stansby, M. E.**: Indust. and Engr. Chem., Anal. ed., **6**, 118, 1934.

(38.) **Lang, K.**: Arch. Exp. Path. u. Pharm., **152**, 366, 1930. (39.) **Leake, C. D.**: J. Pharm. and Exp. Ther., Proc., **33**, 279, 1928. (40.) **Lehman, F.**: Arch. f. exp. Path. u. Pharm., **130**, 250, 1925. (41.) **Luhrig, H.**: (*a*) Chem.-Ztg., **49**, 805, 1925; (*b*) Ibid., **50**, 593, 1926. (42.) **McClure, F. J.**, and **Mitchell, H. H.**: J. Biol. Chem., **90**, 297, 1930. (43.) **McNally, W. D.**: J. Am. Med. Assn., **81**, 811, 1923. (44.) **Marcovitch, S.**: J. Pharm. and Exp. Ther., **34**, 179, 1928. (45.) **Mayrhofer, A.**, and **Wasitzky, A.**: Biochem. Ztschr., **204**, 62, 1929. (46.) **Meyer, R. J.**, and **Schulz, W.**: Ztschr. angew. Chem., **38**, 203, 1925. (47.) **Muehlberger, C. W.**: J. Pharm. and Exp. Ther., **39**, 246, 1930. (48.) **Offerman, H.**: Ztschr. angew. Chem., **3**, 615, 1890. (49.) **Papish, J.**, **Hoag, L.**, and **Snee, W.**: Indust. and Engr. Chem., Anal. ed., **2**, 263, 1930. (50.) **Penfield, S. L.**: Ztschr. anal. Chem., **21**, 120, 1882. (51.) **Phillips, P. H.**, **Hart, E. B.**, and **Bohstedt, G.**: Wisconsin Agr. Exp. Sta. Bull. 123, Oct., 1934. (52.) **Rose, H.**: Ann. d. Chem., **72**, 343, 1849. (53.) **Sanchis, J. M.**: Indust. and Engr. Chem., Anal. ed., **6**, 134, 1934. (54.) **Schulz, H.**: Arch. f. exp. Path. u. Pharm., **25**, 327, 1889. (55.) **Schwyzer, F.**: J. Med. Res., **10**, 301, 1903. (56.) **Scott, E. W.**, and **Henne, A. L.**: Indust. and Engr. Chem., Anal. ed., **7**, 299, 1935. (57.) **Sharkey, T. P.**, and **Simpson, W. M.**: J. Am. Med. Assn., **100**, 97, 1933. (58.) **Sharpless, G. R.**, and **McCollum, E. V.**: J. Nutrition, **6**, 163, 1933. (59.) **Smith, M. C.**, **Lantz, F. M.**, and **Smith, H. V.**: Univ. Arizona Agr. Exp. Sta. Bull. 32, 1931. (60.) **Smith, O. M.**, and **Dutcher, H. A.**: Indust. and Engr. Chem., Anal. ed., **6**, 61, 1934. (61.) **Sollmann, T.**: A Manual of Pharmacology, 3d ed., Philadelphia, W. B. Saunders Company, p. 876, 1928. (62.) **Sonntag, G.**: Ztschr. f. Biol., **41**, 487, 1901. (63.) **Stanton, J. N.**, and **Kahn, M.**: J. Am. Med. Assn., **64**, 1985, 1918. (64.) **Starch, G.**: Ztschr. anorg. Chem., **70**, 173, 1911. (65.) **Starkenstein, E.**, **Rost, E.**, and **Pohl, J.**: Toxicologie, Berlin, Urban u. Schwarzenberg, p. 96, 1929. (66.) **Steiger, G.**: J. Am. Chem. Soc., **30**, 219, 1908. (67.) **Stuber, B.**, and **Lang, K.**: Ztschr. f. klin. Med., **108**, 423, 1928. (68.) **Tappeiner, H.**: Arch. f. exp. Path. u. Pharm., **25**, 203, 1889. (69.) **Treadwell, F. P.**, and **Koch, A. A.**: Ztschr. anal. Chem., **5**, 190, 1866. (70.) **Treadwell, W. D.**, and **Kohl, A.**: Helv. chem. Acta, **9**, 470, 1926. (71.) **Valjavec, M.**: Ztschr. f. d. ges. exp. Med., **85**, 382, 1932. (72.) **Wichman, H. J.**, and **Dahle, D.**: J. Assn. Off. Agr. Chem., **16**, 612, 1932. (73.) **Wieland, H.**, and **Kurtzahn, G.**: Arch. f. exp. Path. u. Pharm., **97**, 489, 1923. (74.) **Wilcox, L. V.**: Indust. and Engr. Chem., Anal. ed., **6**, 167, 1934. (75.) **Willard, H. H.**, and **Winter, O. B.**: Ibid., **5**, 7, 1933. (76.) **Wohler, F.**: Poggendorff's Ann. d. Physik, **48**, 87, 1839. (77.) **Zdareck, E.**: Ztschr. f. d. phys. Chem., **69**, 127, 1910.

A PHARMACOLOGIC STUDY OF TRICHLORETHANOL.

By G. Lehmann, M.D., Dr. Ing.,
FELLOW IN PHARMACOLOGY OF G. D. SEARLE & CO.,
AND
P. K. Knoefel, M.D.,
ASSOCIATE PROFESSOR OF PHARMACOLOGY, UNIVERSITY OF LOUISVILLE SCHOOL OF MEDICINE, LOUISVILLE, KY.

(From the Department of Physiology and Pharmacology, University of Louisville School of Medicine.)

Trichlorethanol is the alcohol corresponding to the aldehyde, chloral. It resembles tribromethanol in structure, but contains chlorine in place of bromine. Trichlorethanol has received little

attention, although it is thought to be formed in the body from chloral hydrate. Its narcotic potency was briefly described by Külz,[3] and Akamatsu and Wasmuth;[1] it has been studied more recently by Molitor.[5] From our earlier studies,[2,4] trichlorethanol appeared to have possibilities for use as a basal anesthetic. We have investigated its anesthetic effect alone and in combination with ether, its action on respiration and circulation, its fate in the body, its action on carbohydrate metabolism and acid-base balance, and on the histology of mucous membranes, heart, liver and kidney.

Trichlorethanol* has been compared with two forms of tribromethanol, crystals† and a solution in amylene hydrate ("Avertin-Fluid," "Avertin with Amylene Hydrate, N.N.R.").‡ Throughout the paper, doses are given in grams per kilogram of body weight. In the case of tribromethanol, this refers to this substance alone, whether used as crystals or the solution in amylene hydrate.

Trichlorethanol is readily handled in the pure state, being liquid above 18° C. Its specific gravity is such that 1 cc. contains 1.55 gm. This is an important difference from "Avertin-Fluid," 1 cc. of which contains 1 gm. of tribromethanol. Trichlorethanol is more soluble in water than is tribromethanol. Table 1 shows its solubility, as determined indirectly with the Abbé refractometer. The greater solubility at lower temperatures prevents trichlorethanol from separating out of solution on cooling, as is the case with tribromethanol. Aqueous solutions of trichlorethanol are more stable than those of "Avertin-Fluid" (Fig. 1), but precautions similar to those taken with the latter should be followed.

TABLE 1.

Temp., ° C.	Grams per 100 cc. water.
0	10.04
20	8.82
25	8.35
37	7.86

1. *Anesthetic Activity.* In depressing the central nervous system, trichlorethanol and tribromethanol are qualitatively and quantitatively similar,[4] compared weight for weight. The effective doses are shown in Table 2. Trichlorethanol appears to have a wider margin of safety, in that it produces sleep and anesthesia in smaller fractions of the fatal dose. This difference has also been noted by Molitor.[5] Death seems to result from respiratory paralysis.

The duration of narcosis and postanesthetic depression with trichlorethanol is slightly greater than with "Avertin-Fluid," and considerably greater than with crystalline tribromethanol (Figs. 2 and 3). The onset of action with rectal administration is slower with

* Prepared by Dr. R. R. Burtner at G. D. Searle & Co. Subsequently we have used some supplied by Merck & Co.

† Prepared by Dr. Burtner.

‡ Winthrop Chemical Company. Purchased.

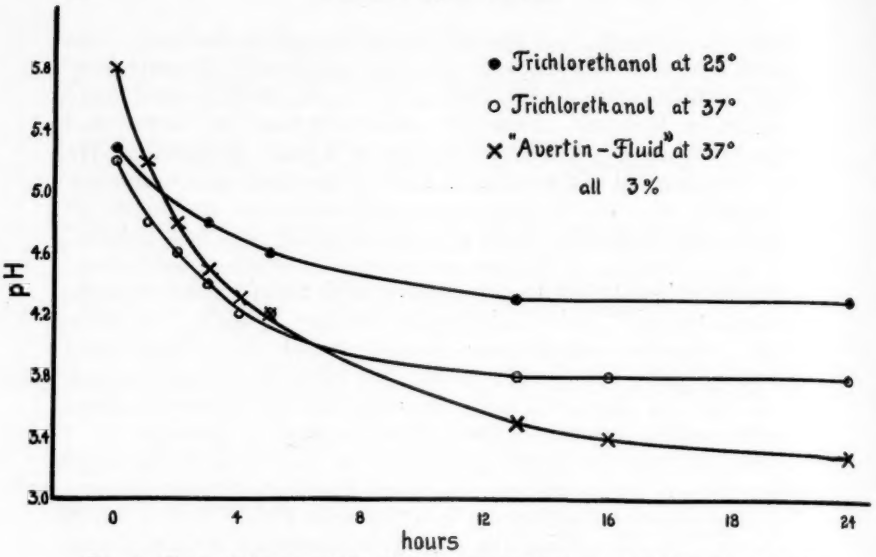

FIG. 1.—Rate of decomposition of 3% aqueous solutions of trichlorethanol and "Avertin-Fluid." Concentration of hydrogen ions determined by the indicator method.

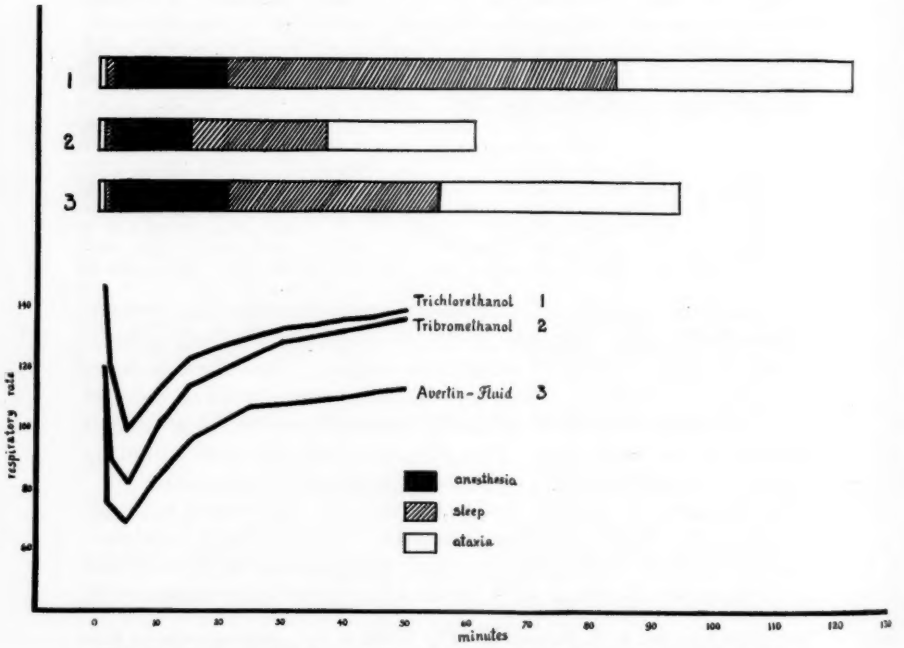

FIG. 2.—Average duration of anesthesia, sleep and ataxia, and respiratory rates in 30 rats, receiving at 1-week intervals intraperitoneal injections of 0.3 gm. per kilo of trichlorethanol, tribromethanol and "Avertin-Fluid" in 3% solutions.

trichlorethanol than with the tribromethanol preparations. The greater duration of action with trichlorethanol is probably to be explained by the fact that in equal weights of trichlorethanol and

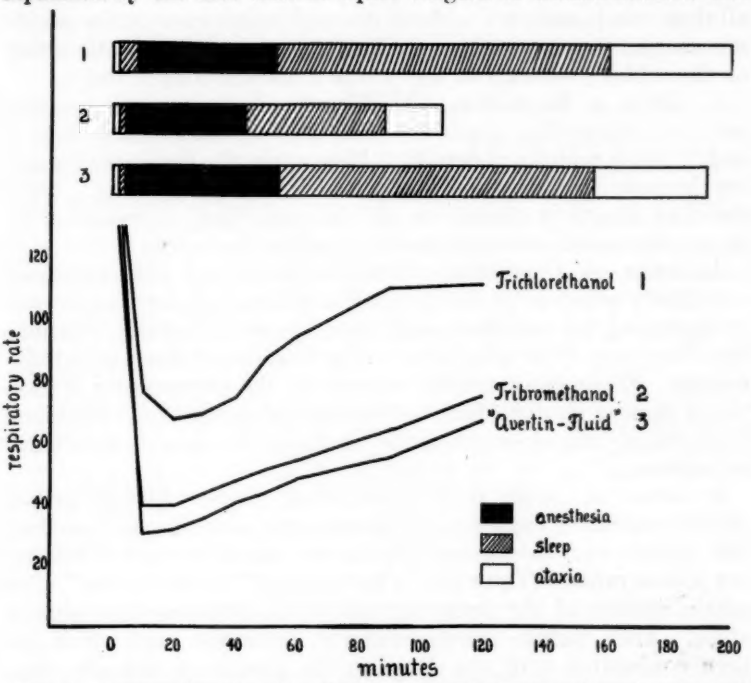

FIG. 3.—Average duration of anesthesia, sleep and ataxia, and respiratory rates after rectal administration of 0.4 gm. per kilo of 3% solutions of trichlorethanol in 14 rabbits, tribromethanol in 7 rabbits and "Avertin-Fluid" in 10 rabbits.

tribromethanol, there are twice as many molecules in the former as in the latter. With "Avertin-Fluid" the amylene hydrate contributes to the narcotic action, so that its duration is about the same as with trichlorethanol.

TABLE 2.

	Trichlorethanol, 3%, gm. per kilo.			Tribromethanol, 3%, gm. per kilo.		
	By mouth in rats.	Intraperitoneally in rats.	Intravenously in rabbits.*	By mouth in rats.	Intraperitoneally in rats.	Intravenously in rabbits.*
Minimum hypnotic dose	0.20	0.10	0.04	0.27	0.10	0.045
Minimum anesthetic dose	0.60	0.15	0.12	0.60	0.20	0.100
Minimum fatal dose	1.00	0.40	0.18	1.00	0.45	0.180

* Duration of injection: 90 seconds.

The analgesic, apart from the narcotic, action is no greater with trichlorethanol than with tribromethanol.

2. *Local Action on Mucous Membranes.* The 3% solutions of all three compounds are without discernible influence on the gastric mucosa and the rectal mucosa. They have a local anesthetic action on the rabbit's cornea, but they cause a marked conjunctivitis.

3. *Action on Respiration.* Trichlorethanol depresses respiratory rate and volume but much less than does tribromethanol (Figs. 2 and 3, and in additional detail[4]). Here again, the presence of amylene hydrate in "Avertin-Fluid" contributes significantly. Since the chief danger in clinical use of "Avertin-Fluid" is serious respiratory depression, trichlorethanol is perhaps preferable.

4. *Action on Circulation.* Trichlorethanol and tribromethanol are equally active in producing a fall in arterial pressure,[4] as a result of depressing the medullary and spinal vasomotor centers. Cardiac function seems to be unimpaired when therapeutic doses are administered. Electrocardiographic records in the experiments of Section 7 showed no disturbance of rhythm or conduction. No histologic change was observed in the hearts of the animals mentioned in Section 8.

5. *Action on Alkali Reserve and Blood Sugar.* Plasma carbon dioxide combining capacity[7] and blood sugar, according to Hagedorn and Jensen, were determined after rectal administration of 0.4 gm. per kilo in rabbits (Table 3). The values of "blood glucose" after administration of the drugs are not to be interpreted as glucose alone. They include glucose and the glucuronic acid which has been conjugated with the drugs in the process of detoxification. The actual blood glucose values cannot be ascertained from these figures. The higher values found after administration of trichlorethanol are explained here also by the difference of molecular equivalents, when the same amount in grams is given.

The alkali reserve is lowered to about the same extent with trichlorethanol and "Avertin-Fluid," as indicated by the average values. In both cases the fall is of minor importance.

6. *Fate in the Body. Tolerance.* As mentioned above, trichlorethanol is detoxified in the body through conjugation with glucuronic acid to give urochloralic acid which is excreted in the urine. The amount of urochloralic acid excreted in the urine of dogs varies, but apparently never reaches the theoretical maximum, if the trichlorethanol were all excreted in this form. It depends probably on the diet of the dogs before the experiment is started, since Quick[6] has shown that a carbohydrate diet increases the amount of glucuronic acid in a normal animal. In 1 case we could isolate 20% of the administered trichlorethanol unchanged.

Also of interest is the rate of excretion. The urine of men receiving 2-gm. doses of trichlorethanol was collected at 90-minute intervals, and 10 cc. of it were acidified and extracted with ether for 4 hours.

After evaporation of the ether and hydrolysis with dilute HCl, the amount of reducing substance was determined by the Hagedorn-Jensen sugar method. Figure 4 shows the results of such an experiment, expressed as glucose equivalents. The excretion remains at a high level for 6 hours and then falls rapidly.

TABLE 3.

Plasma CO_2 combining capacity, vol. %.						Blood sugar, mg. %.								
3% trichlorethanol, 0.4 gm./kilo rect.			3% "Avertin-Fluid," 0.4 gm./kilo rect.			3% trichlorethanol, 0.4 gm./kilo rect.					3% "Avertin-Fluid," 0.4 gm./kilo rect.			
								After injection.					After injection.	
Rabbit No.	Normal.	2 hrs. after injection.	Rabbit No.	Normal.	2 hrs. after injection.	Rabbit No.	Normal.	1 hr.	2 hrs.	3 hrs.	Rabbit No.	Normal.	1 hr.	2 hrs.	3 hrs.
1	47.3	36.9	9	41.9	36.6	1	115	194	281	385	9	114	130	182	195
2	55.1	51.4	10	42.6	34.0	2	107	135	175	192	10	121	132	158	159
3	42.9	35.3	11	48.6	33.8	3	110	226	409	460	11	119	173	233	212
4	46.0	29.8	12	50.2	41.6	4	126	172	177	210	12	121	133	182	196
5	47.0	24.4				5	115	189	186	192					
6	60.0	52.5				6	120	172	176	177					
7	47.1	33.0				7	118	156	274	267					
8	50.4	36.4													
Av.	49.5	37.5	Av.	45.8	36.5	Av.	116	178	239	269	Av.	119	142	189	190

The occurrence of tolerance to trichlorethanol and "Avertin-Fluid" was examined by daily oral administration of 0.6 gm. per kilo to rats. The results are not uniform as Table 4 shows. Some rats seem to have acquired tolerance, particularly to "Avertin-Fluid," some of them seem to have lost it.

TABLE 4.

Rat No.	Duration of loss of posture in minutes.						
	1st day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.
			3% Trichlorethanol.				
1	180	186	180	28	210	200	157
2	250	258	267	325	*		
3	167	240	177	165	173	145	142
4	160	132	137	120	142	139	119
5	196	201	229	251	176	Killed	
6	176	233	240	Killed			
7	224	239	218	227	243	202	236
			3% "Avertin-Fluid."				
8	144	69	*				
9	149	123	110	113	0	41	60
10	189	124	136	101	87	Killed	
11	137	97	13	13	10	26	7
12	200	134	182	*			

* Died during anesthesia.

7. *Action as a Basal Anesthetic.* Four dogs were given rectally 0.2 gm. or 0.3 gm. per kilo of trichlorethanol, and 5 days later 3 of these dogs received corresponding doses of "Avertin-Fluid." Respiratory rate was noted and electrocardiograms taken immediately before and 20 minutes after the administration. Then ether was given by inhalation so that the wink reflex was abolished in about 10 minutes. Arterial blood was then taken and its ether content determined by aëration into standardized chromic acid and iodimetric titration. Ether inhalation was continued until respiration ceased, when blood-ether content was again determined (Table 5). It appears that with trichlorethanol less ether is required than with "Avertin-Fluid."

8. *Histologic Studies.* These were made in three series of animals:

(*a*) Six rabbits receiving by rectum 0.4 gm. per kilo of trichlor-

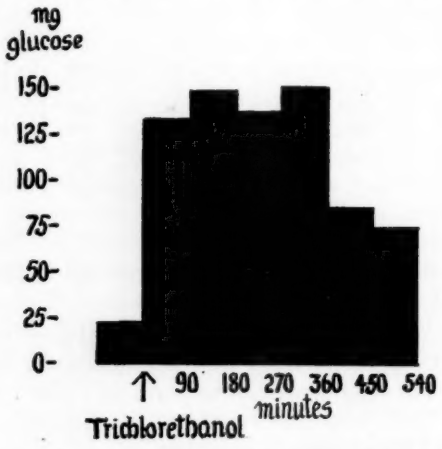

FIG. 4.—Rate of excretion of urochloralic acid in man after oral administration of 2 gm. trichlorethanol.

ethanol in 3% solution, and 2 rabbits receiving the same dose of "Avertin-Fluid" were killed after 24 hours. Heart, liver and kidney were found to be normal.

(*b*) Seven rats received daily 0.6 gm. per kilo of trichlorethanol by mouth, and 5 rats the same dose of "Avertin-Fluid" (Table 4). The surviving animals were killed on the eighth day, and the hearts, livers and kidneys of all the animals were examined histologically. Of the 7 rats receiving trichlorethanol, the livers of 2 (Nos. 1 and 7) showed moderate histologic changes, the one cloudy swelling and fatty degeneration, the other cloudy swelling and atrophy. Hearts and kidneys of these 2 rats were normal. The organs of the rats which had received "Avertin-Fluid" were normal, with the exception of 1 rat, the liver of which showed slight cloudy swelling. The kidney of this rat was severely damaged, with in-

flammatory infiltration in tubuli and glomeruli, some cloudy swelling and necrosis.

(*c*) The effect of trichlorethanol and "Avertin-Fluid" on a previously damaged liver was examined by the following experiment: In 14 rats, liver damage was produced by the oral administration of 1.5 cc. per kilo of a 1 to 1 mixture of carbon tetrachloride and ethyl alcohol. Twenty-four hours later 5 of these rats were given orally 0.5 gm. per kilo of trichlorethanol, and 5 the same dose of "Avertin-Fluid." These rats were highly susceptible to the anesthetics. Four of the rats which had received "Avertin-Fluid" died during anesthesia. Even a higher dose, 0.6 gm. per kilo, does not kill normal rats. Trichlorethanol had less effect. Only 1 of the 5 rats died, but the average duration of the action in the 4 surviving animals was 295 minutes, compared with 135 minutes in normal rats.

The livers of the 4 rats which received the carbon tetrachloride-alcohol mixture alone showed extensive damage near the central veins, with fatty degeneration and infiltration, and areas of recent necrosis, some with hemorrhages. Most of the lobules were involved. The kidneys exhibited small hemorrhages in the glomeruli, focal areas of necrosis, severe cloudy swelling and more diffuse areas of fatty degeneration.

TABLE 5.

Dog No.	3% Trichlorethanol						3% "Avertin-Fluid"					
	Heart rate. Injection.		Respiratory rate. Injection.		Blood ether content, mg. %.		Heart rate. Injection.		Respiratory rate. Injection.		Blood ether content, mg. %.	
	Before.	20 mins. after.	Before.	20 mins. after.	Wink reflex abolished.	Respiration ceased.	Before.	20 mins. after.	Before.	20 mins. after.	Wink reflex abolished.	Respiration ceased.
1. 0.3 gm./kilo rectally	115	125	21	22	65	123	78	90	24	27	138	176
2. 0.2 gm./kg. rectally	130	120	20	24	72	134						
3. 0.2 gm./kg. rectally	92	87	30	34	59	109	69	103	120	280	141	189
4. 0.3 gm./kilo rectally	106	112	22	18	115	149	96	160	47	81	152	171

The administration of the two anesthetics did not increase the extent of the pathologic change already caused by the carbon tetrachloride-alcohol mixture.

Conclusions. Trichlorethanol has an anesthetic action resembling that of tribromethanol and "Avertin-Fluid," but trichlorethanol is less depressant to respiration and has a wider margin of safety. In its action on the circulation, on blood-alkali reserve and on mucous membranes it resembles tribromethanol.

Trichlorethanol is excreted in the urine in conjugation with glucuronic acid and partly unchanged. Tolerance to it is not readily developed.

Trichlorethanol does not produce constant histologic changes in heart, liver or kidney, even after repeated administration. It does not augment a previously existing damage to liver or kidney, but under such circumstances its action is greatly prolonged.

With trichlorethanol used as a basal anesthetic, less ether is required to produce loss of reflexes and cessation of respiration than with tribromethanol.

Trichlorethanol is more soluble in water than is tribromethanol. Its aqueous solutions are more stable than those of tribromethanol.

Trichlorethanol seems deserving of clinical trial.

REFERENCES.

(1.) **Akamatsu, M.**, and **Wasmuth, F.**: Arch. f. exper. Path. u. Pharmakol., **99**, 108, 1923. (2.) **Burtner, R. R.**, and **Lehmann, G.**: J. Pharm. and Exp. Therap., **63**, 183, 1938. (3.) **Külz, E.**: Ztschr. f. Biol., **20**, 175, 1884. (4.) **Lehmann, G.**, and **Knoefel, P. K.**: J. Pharm. and Exp. Ther., **63**, 453, 1938. (5.) **Molitor, H.**: Anesthesia and Analgesia, **17**, 258, 1938. (6.) **Quick, A. J.**: J. Biol. Chem., **70**, 397, 1926. (7.) **van Slyke, D. D.**, and **Neill, J. J.**: Ibid., **61**, 523, 1924.

SIGNIFICANCE OF STANDARD LABORATORY PROCEDURES IN THE DIAGNOSIS OF BRUCELLOSIS.

By E. E. Menefee, Jr., M.D.,

RESEARCH FELLOW IN MEDICINE AND BACTERIOLOGY FOR THE STUDY OF BRUCELLOSIS, DUKE UNIVERSITY SCHOOL OF MEDICINE,

AND

Mary A. Poston,

INSTRUCTOR IN BACTERIOLOGY, DUKE UNIVERSITY SCHOOL OF MEDICINE, DURHAM, N. C.

(From the Departments of Bacteriology and Medicine, Duke University School of Medicine.)

Since 1918, when Evans[4a] demonstrated the close relationship of Bang's B. abortus and Bruce's M. melitensis, there has been in the United States a steadily increasing interest in the disease now known as brucellosis. The great diversity of signs and symptoms produced by this infection compels the clinician to rely upon the laboratory for diagnosis.

Complement fixation as a routine procedure for the diagnosis of brucellosis has been generally discarded, since it offers no advantages over the more simply performed agglutination test. The agglutination test *per se* has not proven to be entirely satisfactory, since various investigators[2,4b,13] have reported proven cases of acute and chronic brucellosis without agglutinins, and Dooley,[3] in a study of 263 students, found agglutinins in 108, only 2 of whom had clinical symptoms of the disease.

Skin tests with different type antigens have been studied by several investigators[1,5,7,12,15] who have generally concluded that the endermic reactions are of aid in diagnosis but have limited value, since patients can often develop an allergic cutaneous reaction without showing symptoms of disease. Moreover, skin sensitivity persists after complete recovery.

It is evident that none of the preceding tests alone, or combinations of them, are to be relied upon in determining the exact immunologic status of a patient. Huddleson,[11] in 1933, devised an ingenious and fairly simple method of determining the opsonocytophagic power of human blood. Using this test, in conjunction with the allergic skin test and agglutinins, he was able to classify all patients into three groups: susceptible, infected and immune.

The object of this paper is to report the result of a survey made by us in an effort to correlate the results of these immunologic procedures with the clinical impressions as determined by history, observations and physical examination. Previous surveys have been done either on groups whose occupational hazard for infection was great, groups who were known to be drinking contaminated milk, or hospitalized patients who were suffering from various known or unknown diseases. We decided to study only healthy individuals who had no known exposure to the disease. We found 1122 male Duke University students who were willing to coöperate, none of whom had any past history of acute or chronic illness suggestive of Brucella infection. Physical examination revealed no abnormalities, and none had complaints at the time of the study. These students came from all 48 states and 7 foreign countries, the majority, however, from the Middle Atlantic and southern sections of this country. In addition to the students used for this survey, we studied carefully a few selected hospital patients, who will be reported at this time since they illustrate some pertinent points.

Technique of Tests. *Cutaneous.* One-tenth cubic centimeter of a 1 to 2000 dilution of brucellergin,[10] a nucleoprotein derived from Brucella organisms, was injected into the skin of the flexor surface of the forearm. The tests were read 48 hours after injection, noting the erythema, edema, and induration. If read before 48 hours, the commonly occurring early false reactions may give an erroneous impression. These false reactions are characterized by inflammation without induration or edema and usually subside rapidly after 18 to 24 hours.

Agglutination. The usual macroscopic method was employed, using serial dilutions of serum from 1 to 20 to 1 to 5120 and a control. The antigen was an equal mixture of *Brucella*, varieties *abortus* 456 and *melitensis* 428. The organisms were grown on liver infusion agar at 37° C. for 2 days. The growths of each strain were washed off in normal physiologic saline, which contained 0.5% formalin and diluted to equal densities. Equal amounts were mixed and the resulting suspension diluted with normal saline to a density of 7 cm. on the Gates apparatus.

Huddleson's[10] rapid agglutination slide test also was used as a check on all sera, but no appreciable differences could be detected.

Opsonocytophagic Test. Huddleson's[10] technique, with minor variations, was followed. Whole blood (5 cc.) was collected in a Wassermann tube containing 0.2 cc. of a 20% solution of sodium citrate in normal saline. The tube was stoppered and gently inverted several times. One-tenth cubic centimeter of the resulting 0.8% citrated blood was removed and placed in a second Wassermann tube. To this was added 0.1 cc. of a heavy (1.0 cm. Gates) normal saline suspension of a 48-hour culture of *Brucella*, variety *abortus* 456. The tube was placed in a 37° C. water bath and was gently shaken at 10-minute intervals. After 30 minutes the tube was removed, the cells were resuspended, and a drop, obtained with a capillary pipette, was placed on a clean polished slide. Smears were drawn in the usual manner. After rapid drying in hot air they were stained by Wilson's method. Fifty segmented neutrophils were counted and a phagocytic index number derived according to the method of Foshay and LeBlanc.[6]

Sedimentation Rate. The sedimentation rate of the R. B. C. was determined by the Wintrobe method.[14]

Results. One hundred and twenty-seven students, or 11.3% of the 1122 students who were skin-tested, gave a positive reaction. It was decided to use these 127 positive reactors for further study and to control the work with 127 students who had negative skin tests.

Of the 127 positive reactors, 114 (89.8%) stated that they drank raw milk in their homes. In comparison, only 46 (36.2%) of the 127 who failed to react gave a history of using raw milk. None in either group had ever had any known contact with infected animals or meat, nor had any ever worked in a laboratory.

The local reactions varied in size from 1.5 by 2 cm. to 8 by 14 cm. Forty-three, or approximately one-third of the group who reacted, had an accompanying lymphangitis; 22 of these complained of tender axillæ, and painful axillary lymph nodes were easily palpable. Twenty-four students had systemic reactions characterized by fever, chills, headaches, myalgia, arthralgia and general malaise. These symptoms usually subsided in 12 to 18 hours and required nothing more than small doses of acetylsalicylic acid. However, 5 students had a fever of 40° C. (104° F.) or higher, and required hospitalization for 3 or 4 days. One student reacted so severely that the skin sloughed over an area of 6 by 4 cm., but the lesion healed spontaneously after a 9-week period. There was no correlation between the amount of local and systemic reactions and the antibody content as evidenced by the opsonocytophagic and agglutination tests. Several students with very marked skin reactions showed little or no evidence of antibodies, while others with only very small local reactions had high antibody titers. All the severe systemic reactions were accompanied by marked local reactions.

The immunologic studies done at the time of reading the skin tests are tabulated in Table 1.

Applying Huddleson's[10] system of classification, 995 (88.7%) of the 1122 clinically well students were susceptible, 116 (10.4%) infected, 6 (0.5%) questionably immune and 3 (0.25%) immune.

Sedimentation rates on the different groups are shown in Table 2.

Goldstein,[8] Heathman[9] and Huddleson[10] demonstrated the appearance of agglutinins after skin testing, and Evans[4c] showed that agglutinins and opsonocytophagic index power are often increased following endermic injections. The results obtained on 20 students with positive skin reactions are in Table 3. The agglutinins and opsonocytophagic reactions slowly decreased after the third week.

TABLE 1.—RESULTS OF IMMUNOLOGIC STUDIES IN 127 SKIN-TEST POSITIVES AND 127 NEGATIVES.

No.	Positive skin test.	Agglutinins.					Opsonocytophagic index.					
		0.	1–20.	1–40.	1–80.	1–160.	0.	1–20.	21–30.	31–60.	61–80.	81–100.
127	127	97	13	10	4	3	2	51	45	18	6	3
127	0	125	2	0	0	0	89	34	3	0	1	0

TABLE 2.—SEDIMENTATION RATE STUDIES.

Group.	Average sedimentation rate, mm. per hr.
127 susceptible students	5.3
116 infected students	4.9
6 questionably immune	5.1
3 immune	5.0

TABLE 3.—EFFECT OF BRUCELLERGIN SKIN TESTS ON AGGLUTININS AND OPSONOCYTOPHAGIC POWER.

Case.	At time of injection.		7 days later.		14 days later.		21 days later.	
	Aggl.	Opsono. index.	Aggl.	Opsono. index.	Aggl.	Opsono. index.	Aggl.	Opsono. index.
1	1–40	74	1–160	90	1–640	92	1–640	98
2	0	26	1–40	24	1–80	40	1–80	44
3	0	18	0	22	1–80	30	1–80	24
4	0	16	1–160	14	1–320	20	1–320	25
5	0	21	0	30	0	42	0	45
6	1–40	26	1–80	34	1–80	46	1–160	44
7	0	20	0	20	0	16	0	24
8	0	18	0	26	1–40	34	1–80	40
9	0	22	0	20	0	30	1–80	32
10	0	21	0	28	0	35	0	39
11	0	24	0	20	0	31	1–80	38
12	1–160	43	1–320	55	1–640	62	1–1280	78
13	0	22	0	44	0	55	1–80	60
14	0	18	0	24	1–80	30	1–160	32
15	1–20	16	1–80	36	1–80	42	1–320	54
15	0	39	0	60	0	68	1–80	85
17	0	28	0	20	0	38	0	61
18	0	14	0	24	1–40	34	1–160	32
19	1–20	21	1–160	36	1–320	40	1–320	44
20	0	19	0	36	0	42	0	50
Average	..	25.3	..	33.1	..	41.3	..	47.4

Another group of reacting students were followed in the same manner except that typhoid vaccine in the usual immunizing doses was given on the 7th, 14th and 21st days (Table 4).

It is evident from these experiments that the endermic injection of brucellergin causes a rise in the patient's opsonins and agglutinins which reaches a peak near the end of the third week. It is also apparent that when additional foreign protein, in the form of killed typhoid and paratyphoid bacilli, are injected in conjunction with brucellergin, the stimulation is greater and more prolonged, the greatest response being reached around the end of the 7th week.

TABLE 4.—EFFECT OF TYPHOID VACCINE INJECTIONS ON IMMUNOLOGIC FINDINGS.

Case.	At time of injection.		7 days later.		14 days later.		21 days later.		28 days later.		35 days later.		42 days later.	
	Agglutinins.	Opso. index.	Agglutinins.	Opso. index.	Agglutinins.	Opso. index.	Agglutinins.	Opso. index.	Agglutinins.	Opso. index.	Agglutinins.	Opso. index.	Agglutinins.	Opso. index.
1	1–20	28	1–40	36	1–80	48	1–320	55	1–1280	61	1–640	67	1–640	68
2	0	20	0	40	1–320	40	1–640	62	1–640	68	1–640	72	1–1280	74
3	0	18	0	34	0	46	0	45	1–40	50	0	55	1–80	63
4	0	21	0	30	1–40	34	0	40	0	40	0	42	0	44
5	1–20	32	1–80	45	1–320	42	1–640	55	1–640	63	1–640	71	1–320	85
6	0	16	0	20	0	33	0	39	0	48	1–320	61	1–640	73
7	0	25	1–80	33	1–640	50	1–640	64	1–1280	74	1–1280	78	1–1280	70
8	0	8	0	18	0	38	0	40	1–160	44	1–320	38	1–160	36
9	1–40	44	1–80	60	1–80	74	1–80	78	1–160	86	1–640	92	1–640	98
10	0	19	0	35	0	38	0	48	0	50	0	54	0	57
11	0	23	0	38	1–40	51	1–80	59	1–80	62	1–160	67	1–320	76
12	0	22	0	18	0	23	0	36	0	64	0	70	0	84
13	0	31	1–80	44	1–160	48	1–320	53	1–640	61	1–1280	68	1–2560	71
14	0	13	0	19	0	33	0	41	1–40	55	1–80	63	1–160	65
15	0	17	0	31	0	30	0	44	0	48	1–320	52	1–320	50
16	0	23	0	22	0	37	0	39	0	55	0	49	0	48
17	1–160	48	1–320	64	1–640	73	1–640	88	1–1280	90	1–1280	92	1–2560	92
18	1–80	74	1–640	88	1–320	66	1–640	78	1–1280	76	1–5120	84	1–5120	96
Av.	..	28.6	..	37.5	..	44.6	..	53.5	..	60.8	..	65.2	..	69.4

Dooley,[3] in 1932, pointed out that fever due to any cause might increase serum agglutinins against Brucella whether there had or had not been a previous clinical infection with Brucella. This was confirmed by our studies of the 9 students who became ill at various times with proven streptococcic sore throat. Their immunologic reactions toward Brucella are expressed in Table 5.

TABLE 5.—EFFECT OF INTERCURRENT STREPTOCOCCAL SORE THROAT ON IMMUNOLOGIC FINDINGS.

Case.	Three weeks prior to illness.		During illness.	
	Agglutinins.	Opsono-cytophagic-index.	Agglutinins.	Opsono-cytophagic index.
1	0	18	1–160	39
2	0	14	1–80	50
3	0	27	1–320	53
4	0	0	1–160	43
5	1–20	28	1–640	76
6	0	5	1–320	47
7	0	17	1–80	39
8	1–20	31	1–1280	88
9	0	23	1–80	64

It appears from this that an unrelated febrile illness may cause not only a rapid increase in serum agglutinins, but also a corresponding increase of opsonocytophagic power against Brucella in patients with no previous clinical infection with Brucella.

It is a well-known immunologic fact that one may be immune to a disease and yet show no circulating antibodies to the specific antigen. Typhoid fever is a classic illustration of this, and patients who have recovered may not have circulating immune bodies. However, if injections of bacilli or their components are given to such a patient, there is usually a rapid rise of measurable antibodies. It is possible that this principle applies to Brucella infections. If, during a survey, the immunologic factors are measured at their peak, a much more accurate picture should be obtained. All students, therefore, who had received merely the endermic brucellergin injection were recalled at the 3d week. The ones who had also been inoculated against typhoid fever were called back at the end of the 7th week. The results of the studies on both groups done at these times are shown in Table 6.

TABLE 6.—INCREASE IN NUMBER OF IMMUNE RESPONSES PRODUCED BY INJECTIONS OF BRUCELLERGIN AND TYPHOID VACCINE.

	Agglutinins.						Opsonocytophagic index.					
No.	0.	1–20.	1–40.	1–80.	1–160.	1–320.	0.	1–20.	21–30.	31–60.	61–80.	81–100
127 . . .	29	5	7	16	19	51	0	6	15	49	38	19

When these figures are analyzed it is evident that there has been a rather marked change. The 116 who originally had been classified as infected were now reduced to 70. Those questionably immune had risen from 6 to 38, and the number of immunes from 3 to 19.

These latter studies agree much more closely with the clinical impression than those made earlier. However, it is hard to correlate the fact that 70 students who, from a clinical standpoint, are perfectly well, would be classified as infected by the accepted standards of classification. It must be concluded that most strains of Brucella in this country are relatively avirulent or that the infecting dose is very small. In any case, it appears that too much weight should not be given to laboratory findings alone, and that their interpretations must be very guarded. One is probably justified in saying that the patient is infected, but one cannot say that the patient has brucellosis.

The following short summaries of 4 hospital patients illustrate the misleading nature of established laboratory findings.

Case Histories. No. 95169. A 23-year-old graduate student entered Duke Hospital complaining of afternoon fever and chilly sensations of 4 days' duration. Physical examination except for a temperature of 39° C.

(102.3° F.) was essentially negative. Brucellergin skin tests were positive. He had serum agglutinins 1 to 160 and an opsonocytophagic index of 37. W. B. C. 4300, with relative lymphocytosis. On the third hospital day he developed sore throat and slight generalized enlargement of the lymph nodes. His W. B. C. rose to 18,000 with 26% monocytes, and his heterophile antibody titer was 1 to 1024. Two weeks after recovery and discharge he returned, complaining again of fever, general malaise, and slight diarrhea. Physical examination was essentially negative, and the W. B. C. was 4800. *Salmonella schottmülleri* was isolated from the stools, and he made an uneventful recovery. This is an example of infectious mononucleosis, followed by paratyphoid fever, which could have been diagnosed incorrectly Brucellosis by the laboratory findings.

No. 92654. A white male, aged 43 years, entered the hospital complaining of general lassitude, weakness, and low-grade fever of 10 months' duration. Brucellergin skin tests were positive, agglutination positive for *Brucella* in a dilution of 1 to 80, and the opsonocytophagic index was 43. Proctoscopic examination revealed a small rectal polyp which was removed. Pathologic study revealed an adenocarcinoma.

No. 77127. A 26-year-old white male entered the hospital complaining of intermittent fever for 11 months. Brucellergin skin tests were negative; there were no demonstrable agglutinins and his opsonocytophagic index was 0. There was generalized enlargement of the lymph nodes. One of these was removed, and *Brucella*, variety *suis*, was isolated. This patient subsequently died, and the diagnosis of Brucellosis was proven at necropsy. This instance of chronic Brucellosis would not have been correctly diagnosed had complete dependence been placed in the usual diagnostic procedures.

No. 50291. A 31-year-old white male entered the hospital complaining of fever, chills, and a sore throat for the past 48 hours. Physical examination revealed unusual ulcerations in the mouth and pharynx. Skin tests with Brucellergin were negative; there were no agglutinins and the opsonocytophagic index was 0. *Brucella*, variety *melitensis*, was isolated from the blood stream on the second hospital day. He responded well to immune sera and after 9 days developed an agglutinin titer of 1 to 320. His opsonocytophagic index was then 92. The Brucellergin skin test is still negative after 4 months.

Summary and Conclusions. 1. There appears to be a direct relation between raw milk consumption and skin reaction to brucellergin.

2. Skin tests are not without danger, since severe local and systemic reactions are not infrequent.

3. There appears to be little correlation between skin reactivity and measurable antibody content of the serum.

4. Endermic injection of brucellergin will cause a definite increase of circulating antibodies in allergic patients.

5. Brucellergin followed by typhoid vaccine will cause a greater and more prolonged increase of circulating antibodies than brucellergin alone.

6. Any febrile reaction will cause an increase of circulating antibodies.

7. In survey work, a better immunologic picture is obtained by measuring the antibodies at the height of the antibody response to brucellergin.

8. Negative skin tests, agglutinins and opsonocytophagic reactions do not rule out the possibility of Brucella infection.

9. Positive skin tests, low agglutinins and a moderate opsonocytophagic reaction, even when accompanied by suggestive signs and symptoms, do not prove the diagnosis of brucellosis.

REFERENCES.

(1.) **Amoss, H.**, and **Leavell, H.**: Arch. Int. Med., **48**, 1192, 1931. (2.) **Carpenter, C. M., Boak, R.**, and **Chapman, O. D.**: J. Immunol., **17**, 65, 1929. (3.) **Dooley, P.**: Arch. Int. Med., **50**, 373, 1932. (4.) **Evans, A. C.**: (*a*) J. Infect. Dis., **22**, 576, 1918; (*b*) J. Am. Med. Assn., **103**, 665, 1934; (c) Pub. Health Rep., **52**, 1419, 1937. (5.) **Favorite, G. O.**, and **Culp, C. F.**: J. Lab. and Clin. Med., **20**, 522, 1935. (6.) **Foshay, L.**, and **LeBlanc, T. J.**: Ibid., **22**, 1297, 1937. (7.) **Giordano, A. S.**: J. Am. Med. Assn., **93**, 1957, 1929. (8.) **Goldstein, J. D.**: J. Clin. Invest., **13**, 209, 1934. (9.) **Heathman, L. S.**: J. Inf. Dis., **55**, 243, 1934. (10.) **Huddleson, I. F.**: Brucella Infection in Animals and Man, New York, The Commonwealth Fund, 1934. (11.) **Huddleson, I. F., Johnson, H. W.**, and **Hamann, E. E.**: Am. J. Pub. Health, **23**, 917, 1933. (12.) **Levin, W.**: J. Lab. and Clin. Med., **16**, 275, 1930. (13.) **Simpson, W. M.**: Ann. Int. Med., **4**, 238, 1930. (14.) **Wintrobe, M. M.**, and **Landsberg, J. W.**: Am. J. Med. Sci., **189**, 102, 1935. (15.) **Yeckel, H. C.**, and **Chapman, O. D.**: J. Am. Med. Assn., **100**, 1855, 1933.

SHORT WAVE AND ULTRA SHORT WAVE DIATHERMY.

By Heinrich Brugsch, M.D.,
INSTRUCTOR IN MEDICINE, TUFTS COLLEGE MEDICAL SCHOOL,
AND
Joseph H. Pratt, M.D.,
CLINICAL PROFESSOR OF MEDICINE, TUFTS COLLEGE MEDICAL SCHOOL,
BOSTON, MASS.

(From The Short Wave Department of the New England Medical Center.)

By the term short wave diathermy or short wave therapy is meant the therapeutic use of an electric or electromagnetic field produced by high frequency currents of a wave length ranging from 3 to 30 meters. To waves from 3 to 10 meters in length the term ultra short waves is often applied. The designation short wave therapy is rather unfortunate as it may occasion confusion with short wave radiations such as ultra violet and Roentgen rays. Our present knowledge began with d'Arsonval's[4] discovery that high frequency currents can be applied to the human body without danger of shock and with the disappearance of muscular irritability with increasing frequency. It was also d'Arsonval who first attempted to use them in treatment. Increased output of energy was achieved by improvements in construction of machines. The technical foundation of short wave therapy was laid chiefly by Esau[8] and Scheres-chewsky.[27b] The first demonstration of the usefulness of these short waves in treatment was made by Schliephake[28b] who exposed a painful furuncle on his own nose to their action with the result that the pain was at once allayed and the inflammation quickly subsided.

An increasingly large number of publications have appeared since

Schliephake[28a] published his first papers in 1928. Books in German, French, Italian and English with contributions in medical journals totaling, according to de Cholnoky,[5a] approximately 750 publications indicate the growing interest in this subject. Unfortunately, many communications display an unjustifiable optimism regarding the value of short wave therapy. This is usually the result of drawing conclusions from an inadequate number of observations, and of attributing all recoveries to the treatment employed.

To enumerate all diseases and symptoms for which short wave therapy has been regarded of value would almost cover the whole field of medicine.

There are, however, conditions which make it difficult to evaluate the results of short wave therapy even for those who are working in this field. First of all there is no method available for measuring the amount of energy absorbed by the part or parts of the body under treatment. Recently, Mittelmann[22] demonstrated a new device which at least enables one to measure the amount of energy absorbed by the whole body, but it still leaves unsolved the problem of how much energy a particular area of tissue is getting. The inability to measure the output or the energy utilized makes difficult the comparison of the results obtained by different authors. Other obstacles are that differences in the size of electrodes, their application, their distance from the part of the body treated, the length of cables and the "tuning in" of the machine, which alter the output or the configuration of the condenser field. These difficulties are further increased by the fact that machines of different size and construction with varying outputs of power, with and without the condenser field, are in use in this country and in Europe. When improvement consists of the removal of subjective symptoms it is often difficult to determine whether this is due to the action of the short waves or to the influence of suggestion, always an important factor when a new treatment is given, and especially so when it consists of radiations generated by an impressive looking machine.

Haase and Schliephake,[11] and Liebesny, Wertheim and Scholz[20] concluded on the basis of numerous experiments that short waves have a specific bactericidal effect in addition to their thermal action. Schereschewsky and Felton,[27a] in unpublished work and, more recently, Ruete[26] and a number of other investigators (cited by Krusen[17]) were unable to demonstrate any specific action on bacteria. Experiments by Pflomm[23] seemed to prove that the short wave produced specific action on the circulation of blood through the capillaries and on the autonomic nervous system, but recently his conclusions have been overthrown by Weiss, Pick and Tomberg[29] who show the changes produced in the blood vessels are due entirely to the thermic effects. It can be definitely asserted that heat production in the tissues is the only action of short waves that has been demonstrated.

The chief advantage of the short waves over the much longer waves employed in conventional diathermy is that they penetrate the tissues much more, and generate higher temperatures at a considerable depth below the surface of the body.

Even the so-called "athermic therapy," recently employed and endorsed by the Austrian authors Liebesny[19d] and Weissenberg,[30] can probably be explained as due to the production of a mild, insensible heat, the effect of which is possibly more favorable in certain conditions than strong heat. If the current is so weak as to be really "athermic" it probably has no therapeutic action at all. Hasche[12] has been able to study separately the "thermic" and the "athermic" short wave field and concludes that the latter has no bactericidal power and no physiological effect.

During the past 4 years the effect of treatment with ultra short and short waves has been observed at the Boston Dispensary on a variety of pathologic conditions. It is hoped that an analysis of the observations made on more than 600 patients treated will be of help in determining the value of short wave therapy in some diseases, its lack of value in others, and its effectiveness compared with other forms of treatment.

Methods. Three different types of machines have been used: First, a spark-gap apparatus generating a band of waves ranging in length from about 10 to 30 meters. With this, rubber electrodes applied to the skin were used. Second, an apparatus with two large vacuum tubes. This was the most powerful generator obtainable. It has an output which reaches 800 watts on a 15-meter wave, 600 watts on a 6-meter and 300 watts on a 4-meter wave. With it, wave lengths from 3.5 to 7 meters may be used and also a fixed wave length of 15 meters. As this machine was the same as that employed by Schliephake,[28d] Liebesny[19c] and other European workers, failure to confirm the curative effect obtained by them in some diseases cannot be attributed to any deficiency in the equipment employed. Third, a small vacuum tube machine with an output of 225 watts on a wave length of 6 meters.

The glass shoe electrodes designed by Schliephake have been found most satisfactory as with them it is possible to vary easily and rather accurately the distance of the electrode from the surface of the skin. With them the application of short waves can be supervised better than with any other form of electrodes. Less danger of burning attends their use, as an air gap is left between the electrodes and the skin. Especially designed electrodes for rectum, vagina and axilla are available.

Important variations in the thermal effects at various distances below the surface of the skin depend on the distance of the electrodes from the skin and the arrangement of the two electrodes to form the condenser field.

Different wave lengths, namely 4, 6, 7, and 15 meters, have been used and the results compared to determine if there exists an optimum wave length for different conditions. This attempt has failed and it is impossible to state that a certain wave length is the best in the treatment of a particular disease. Knowing that the output of power decreases when the very short waves are employed, it was found in accordance with the experience of others that, in conditions where no strong heat effect was desired, low wave lengths were usually to be preferred.

As, theoretically at least, ultra short waves should generate more heat at a greater depth than do the longer waves, we used the 4 or 6 meter in treating deep seated lesions, such as lung abscesses and empyema. Coulter and Carter[2] and Coulter and Osborne[3] have shown experimentally that longer wave lengths raise the temperature of the deeper tissue to as high a point as the 6-meter wave. Although they used machines with less output than ours, it is possible the same result would have been obtained with the equipment and technique we employed. In certain cases in our experience on patients where a high degree of heat was important, it seemed as if better results were obtained with a 15-meter wave than with a 4 or 6 meter. This can probably be explained as due to the greater electrical energy when the 15-meter wave length is used.

The following case illustrates the injurious effect of an excess of heat. The patient was a 46-year-old male who had recurrent furuncles. On 3 occasions cure was quickly effected by 2 treatments, each lasting 10 minutes. When he returned a fourth time, after an interval of many months, the attempt was made to cure a small furuncle that had appeared on his wrist by a single treatment of 20 minutes' duration. The result was necrosis of the furuncle and the surrounding tissues. Ulceration occurred and delay in healing.

When a definite heat effect was sought, the duration of the treatment was usually 20 minutes. On the other hand, if a mild hyperemia was desired, a treatment lasting only 5 or 10 minutes was given. The initial treatment in many cases was limited to 10 minutes even when a rather intense generation of heat in deeper tissues seemed desirable. One treatment a day was the rule; occasionally in dealing with severe inflammatory lesions a second treatment was given the same day. Rarely an unfavorable reaction occurred, either in the form of an increase of pain, faintness or evidence of vasomotor weakness. When this took place treatment was omitted the following day. Thereafter the treatments were resumed without untoward symptoms.

As Rausch[25] and de Cholnoky[5a] have observed, a slight fall of blood pressure and increase of blood sugar occurs during short wave treatment. These transitory effects, however, did not interfere with the treatment of our arteriosclerotic patients. Although age is no contraindication for short wave treatment, more care is necessary in elderly patients regarding duration and intensity of treatment since they are more liable to the unfavorable reaction just mentioned. It has never been necessary, however, to abandon the treatments. There is no difficulty in dealing with the young, as children react especially well. Our youngest patient, a baby of 6 months with a large abscess covering one-third of its back, was well on the road to recovery within a week.

To have a check on the results, the dispensary patients were examined in the various clinics from which they were referred, not only before treatment, but during its progress and at its close. Helpful in judging results in infections were reduction in the leukocyte count and especially in the sedimentation rate.

Excepting a slight burn in one case, already mentioned, there were no untoward results. To avoid burns and other injurious effects, short wave therapy was refused to patients presenting any of the following conditions; (1) impaired sensation of the skin; (2) arterial

or venous occlusion of peripheral vessels with evident damage to the tissues; (3) advanced stages of diabetes mellitus unless under strict hospital supervision; (4) arteriosclerosis with marked hypertension.

Pregnancy is not a contraindication and studies have shown there is no danger of injuring the fetus (Raab and Hofmann[24]). During menstruation, short wave treatments should be discontinued since there may be increased flowing as a result of hyperemia of the uterus produced by the short wave.

Results. *Furuncles, Carbuncles and Abscesses.* Schliephake has shown that acute suppurative lesions of the skin and subcutaneous tissues are quickly healed by short waves. He states in the latest edition of his book[28e] that he treated over 500 cases of furunculosis with only one failure. In all the other cases, healing took place in a very short time while patients continued at work. Very favorable results were also obtained by Liebesny[19a] of Vienna."In about 500 acute inflammatory lesions" Liebesny observed only 1 case in which a definite exacerbation occurred following ultra short wave treatment and 1 case with delayed healing. He had striking success in treating that serious condition, furunculosis of the face, reporting only 2 deaths in 86 cases. In this country, excellent results in a large series of cases have been reported by Egan[7]. de Cholnoky[5c] cured 57 cases of furunculosis, and only in 1 case was an incision made.

The least enthusiastic opinion we have seen regarding the treatment of furuncles and carbuncles with the short wave from one who has had experience with adequate apparatus and had used proper technique is that of Kowarschik.[16a] He admits its superiority to other methods of applying heat; first, as the short wave acts at a greater depth and, secondly, it causes no maceration of the surrounding tissues which favors the spread of the inflammatory process, as do moist compresses and poultices. For treating simple infections he thinks the rapidity of cure does not compensate for the expense. He insists that no effective method is absolutely harmless and points out the danger of causing a spread of the infection if an improper technique is used as Haas and Lob[10] found. Kowarschik[16a] states the undoubted truth that every severe infection of the subcutaneous tissues should be under the supervision of an experienced surgeon, in addition to the short wave specialist, and the former should decide when short wave treatment should be interrupted and an operation performed.

There is a general agreement among those who have had experience with short wave therapy that pain in the infected areas quickly disappears. Usually one or two short exposures are sufficient to give speedy relief from tension and throbbing. If treatment is begun in the early infiltrative stage, regression may occur. In more advanced cases, especially where pus has formed, spontaneous rupture of the abscess usually takes place.

This form of treatment has a great advantage over other procedures in dealing with furuncles of the upper lip, nose and cheeks. Operative procedures have yielded a shockingly high mortality. The best surgical practice of today in dealing with these dangerous lesions is reflected in the recent paper by Maes[21] on this subject, who advises complete rest to the parts. Talking and chewing should be forbidden when the lip is involved. In the statistics of Küttner-Dittrich,[18] the mortality of furuncles of the upper lip was 19%, while in Koslin's[15] series it was 30%. Since direct local application of heat to the edematous tissues is difficult, the employment of the glass shoe electrodes with their air gap between skin and electrode is especially advantageous.

We have treated 51 cases of furuncles. Eight of these involved the upper lip, nose or cheek. One of these was a severe infection. Swelling of the nose with edema of the right half of the upper lip and discharge from the right nostril developed. The next day he was given a short wave treatment. During the following night, edema of the lip increased and he had difficulty in breathing. The temperature rose to 100.2°. He was given a second treatment and put to bed. He recovered with only those two treatments. Four of the 51 are put down as failures as they abandoned treatment, but it is possible they felt so little discomfort that they did not consider further treatment necessary. In the other 47, there was rapid improvement and healing. There was no fatality. The duration of a single treatment was usually 10 minutes but occasionally two treatments were given daily.

In agreement with Schliephake, Liebesny and others, we have obtained good results in the treatment of carbuncles. The treatment failed in 2 cases. One of these, a 60-year-old man with severe diabetes, had septicemia when treatment of an extensive carbuncle started. The other, a large carbuncle of the back of the neck, was incised after 3 treatments by a surgeon who preferred the knife to conservative treatment. Healing was speedy. In 9 of our 11 cases, there was a rapid localization of the inflammation, with the formation of pus in one area, decrease of pain, and spontaneous rupture with good drainage. In our series, the average duration of treatment was 12 days; average number of treatments, 9. In every one of these 9 cases it was possible to prevent surgical intervention with its large incision, prolonged suppuration, slow healing and unsightly scar formation.

Paronychia, especially its chronic form, responded well to short wave therapy. In 8 of 10 cases with chronic infiltration and continuous drainage of pus which had persisted for from 2 months to a year or longer in spite of a variety of local treatments, improvement followed the use of short wave; suppuration ceased and there was decreased swelling. The other 2 cases were not followed sufficiently long to permit of a definite conclusion. One of these took only 3 treatments, the other only 4. The average number of treat-

ments in those successfully treated was 11. de Cholnoky[5d] and Egan,[7] in this country, also obtained good results in this disease with the short waves.

Lymphadenitis. In cases of lymphadenitis of unknown etiology or following the extraction of teeth, our results were poor. In only 3 of 12 cases did the swelling of the lymph nodes subside. In all cases, however, subjective symptoms were relieved, but in 3 of these an incision was necessary.

Arthritis. Good results were obtained in *acute arthritis.* This is in accord with de Cholnoky's conclusion that short wave therapy gives the best results in the acute and subacute inflammations of the joints in which long wave diathermy can be used only with extreme caution and even then with the danger of undesirable reactions. In 7 patients with *gonorrheal arthritis,* there was rapid and marked improvement. Pain subsided speedily. Four were practically cured. Two in whom there was chronic involvement of the joints had less pain but no objective improvement. Our results correspond with those previously reported by many investigators. No other method of treatment is so successful. de Cholnoky, after making a study of the literature, states that the results are uniformly satisfactory.

Fourteen patients with acute infectious arthritis of unknown origin did well. In these the swelling subsided after 3 or 4 treatments and motion was restored after about 2 weeks. Average total duration of treatment was 20 days. Three cases showed no improvement. The effect of treatment was especially striking in the case of recurrent acute arthritis of the right elbow joint in a 55-year-old man. The elbow was greatly swollen and acutely inflamed. A roentgenogram revealed marked destruction and a tumor-like proliferation of the cartilage (chondroma). The blood sedimentation rate was 12 mm. in 1 hour (Westergren) before treatments were started and 3 mm. in 1 hour when discharged. He was treated in 2 attacks. In both there was rapid and complete subsidence of the acute inflammation. Pain ceased after 2 treatments and motion was restored after 4 treatments.

In the treatment of *chronic osteo-arthritis* (hypertrophic arthritis), a disease in which it was thought this new form of treatment would be most beneficial, the reports are conflicting. In the third edition of his textbook, Schliephake[28f] speaks more reservedly of the benefits of local ultra short wave treatment than in the first edition. In a later publication[28g] dealing with joint diseases, he recommends short wave electropyrexia when a large joint or multiple joints are involved and, in addition, the use of drugs and other therapeutic methods. In the first edition of his textbook, Schliephake[28c] gives the impression that practically all patients are benefited by placing the affected joints in a condenser field, but in his most recent paper[28g] he states that often no improvement is obtained by local treatment.

Kowarschik[16b] has obtained good results with short wave therapy but not essentially better than with the older forms of thermotherapy. Horsch[13] treated a large series of cases of hypertrophic arthritis and obtained improvement in only 23%. On the other hand, de Cholnoky[5b] treated 35 cases with only a few failures. Usually after a few treatments pain was diminished, swelling decreased and functions improved.

Fourteen of our 44 cases of hypertrophic arthritis showed some slight improvement. Many of these had marked changes in the joints and bones and therefore less pain, and better motion was all that could be expected.

Rheumatoid Arthritis. Contrary to the experience of most investigators, we had more success in atrophic than in hypertrophic arthritis. Among 38 patients with rheumatoid arthritis, in 20 the pain was lessened and better function of the joint obtained. In 3 of 7 cases of *spondylitis ankylopoietica* some improvement was noted. An average number of 10 treatments was given these patients.

Rhinitis. Short wave therapy has been given to a number of cases of *acute rhinitis* occurring in members of the medical staff and employees of the hospital and dispensary. A quick subsidence of symptoms was observed in every case after an early short wave treatment, but there was a recurrence in some cases within a few hours.

Vasomotor rhinitis due to allergic conditions did not subside during short wave treatment.

Empyema of the Accessory Nasal Sinuses. The beneficial effect of short wave therapy in *acute* and *chronic inflammation of the accessory nasal sinuses* has been as striking in our experience as in that of earlier workers in this field. All are agreed as to its value. Often occlusion of the nasal passages is relieved after the first treatment. In cases that respond favorably, the discharge quickly lessens and loses its purulent character. Even in cases of long duration the discharge may entirely disappear or be reduced to an insignificant amount. Recurrence of symptoms within a few months often occurs. Many of our patients had been previously subjected to various operations. A number of patients upon whom operations had been advised were freed from their symptoms by short wave treatment. In our series of 80 cases, no effect on the nasal discharge was obtained in 26, while in 42 the discharge was lessened or lost its purulent character or ceased. The subjective symptoms, headache, sense of fullness, and so on, disappeared under treatment in all but 11 cases. One patient with severe chronic sinusitis failed to improve when treated in a 4-meter condenser field. A year later he obtained signal benefit from a second course of treatments in which the 6-meter wave was used. The best results have been obtained in those cases in which the maxillary antra were involved. The average duration of treatment was 3 weeks. The number of

treatments usually ranged from 7 to 18. The length of exposure to the short wave was generally 15 to 20 minutes.

Prostatic Disease. In 7 cases of *hypertrophy of the prostate* and 3 of *chronic prostatitis* in which frequency of urination and pain were outstanding symptoms, there was marked relief obtained from ultra short wave radiation. Usually during treatment urination decreased from many times a day to the normal number. After treatment was discontinued the symptoms recurred.

Bursitis. Of 72 patients with *subdeltoid* or *subacromial bursitis* 35 obtained symptomatic relief from short wave therapy, and 9 were completely cured. Roentgenograms showed a diminution of calcified deposits in 3 patients. Prolonged treatment was required as a rule, which in a few cases lasted 2 months. Occasionally, a reaction followed the first 2 or 3 treatments.

Neuritis and Neuralgia. In 1 case of severe acute sciatica which was treated in our hospital there was rapid cessation of pain and no recurrence after treatment. Ambulatory patients with sciatica on the whole did not do well. In some patients there was a definite hypersensitivity even to slight heat. In contrast to the favorable results obtained by de Cholnoky we failed to obtain improvement in 3 cases of trigeminal neuralgia and 3 cases of occipital neuralgia.

Myositis and Myalgia. In painful affections of the muscles of unknown etiology lessening of pain and restoration of function usually took place rapidly.

Ulcerations Following Radium Therapy. Three cases have been successfully treated. In the first patient a year after a cancer of the upper lip had been removed by radium an ulcer formed in the poorly vascularized tissues. As it showed no tendency to heal excision was considered, but it was decided to try first the effect of short wave. When treatment was instituted, the lesion measured 15 mm. by 9 mm. surrounded by an erythematous area of equal width. There was less tenderness after 2 treatments. After 3 treatments the size of the ulcerated area was 10 by 8 mm. After 5, it measured only 8 by 4 mm. Treatment was then discontinued. A few days later, healing was completed.

After radium treatment of 2 large cancers of the tongue, deep extensive ulcers formed which showed no tendency to heal. In both there were sloughing ulcers involving the whole left border of the tongue. Daily treatments of 20 minutes' duration were given. The electrodes were placed on each side of the mouth at about 3 cm. distant from the cheek. A wave length of 6 meters was used. In 1 case, sloughing and pain decreased after a few treatments and healing was completed in 2 months. In the second case, the ulcer was treated by various means for over a year without success. Pain was constant and severe. Eighteen short wave treatments were given before healing began. A month later only slight ulceration remained. After healing the tongue was smooth and clean but a deep wedge-like defect marked the site of the ulcer.

Lung Abscess. It was the remarkable results reported by Schliephake in the treatment of this serious and often fatal disease that was the chief reason for the importation of the expensive powerful short wave apparatus which he employed. It seemed reasonable that if we hoped to duplicate his results we should possess the same equipment and use the same technique. Schliephake[28d] reported 9 cases of lung abscess, all of which were cured by short wave. The evidence from case histories was supported by roentgenograms. Liebesny[19b] claimed 4 cases were cured among 6 treated. The 2 failures were attributed to faulty technique. Fiandaca[9] reported the successful treatment of 10 of 12 cases, but there was no report of the condition of the patients after leaving the hospital. Cignolini[1] treated 7 cases with varying results, "*esito vario,*" and reported 2 cases treated by colleagues that were cured. Izar[14] treated 3 cases of lung abscess 20 to 40 minutes daily for a month, using 4, 8 and 15-meter waves, with no result. Further confirmation of the value of short wave in the treatment of lung abscess was apparently furnished in a recent report by Dieker[6] from the Heidelberg medical clinic of 15 recoveries of 16 cases treated. This paper is illustrated with Roentgen ray evidence of cures effected. In spite of the fact that all the reports in the literature in which the Ultra-Pandoras was used were favorable, we, using the same machine, have not cured a single case of lung abscess among 8 treated. In 1 case, the clinical results were striking. The Roentgen ray, however, showed a persistence of the abscess although much diminished in size. Within a year symptoms returned and at operation a large abscess was found.

In the treatment of bronchiectasis, bronchial asthma and chronic bronchitis, we have seen no definite improvement.

We have had only failures in the 1 or 2 cases treated of the following diseases: multiple sclerosis, syphilis of the central nervous system, subacute bacterial endocarditis and osteomyelitis.

Two of our 3 cases of Raynaud's disease were treated without success. Even a mild treatment increased pain. Both cases showed definite organic changes in the fingers. In the third patient, the relation of the spastic attacks to emotional disturbances was undoubted. Although gangliectomy had been advised in this case, a a good result was obtained by short wave therapy. As the psychic element was important in the causation of symptoms, it may have been the chief factor in the therapeutic success.

Summary. In our experience, short wave diathermy hastened cure in acute subcutaneous infections (furuncle, carbuncle, abscess). Good results, often cure, were obtained in certain forms of acute arthritis, especially that of gonorrheal origin. Acute and chronic bursitis were often favorably influenced. Symptomatic relief occurred in about half of the cases of rheumatoid arthritis and in exacerbations of chronic sinusitis.

In the treatment of lung abscess and bronchiectasis, no favorable effect has been obtained by us.

In conclusion, the statement is warranted that short wave therapy is safe and easy of application, and of definite, although limited, therapeutic value.

REFERENCES.

(1.) **Cignolini, P.**: Marconiterapia, Milano, Ulrico Hoepli, p. 300, 1936. (2.) **Coulter, J. S.**, and **Carter, H. A.**: J. Am. Med. Assn., **106**, 2063, 1936. (3.) **Coulter, J. S.**, and **Osborne, S. L.**: Ibid., **110**, 639, 1938. (4.) **D'Arsonval, J.**: Compt. rend. Soc. de biol., p. 318, May, 1891. (5.) **de Cholnoky, T.**: (*a*) Short Wave Diathermy, New York, Columbia University Press, 1937; (*b*) Ibid., p. 194; (*c*) Ibid., p. 217; (*d*) Ibid., p. 225. (6.) **Dieker, W.**: Deutsch. med. Wchnschr., **63**, 1076, 1937. (7.) **Egan, W. J.**: Arch. Phys. Ther., *X*-ray and Radium, **17**, 688, 1936. (8.) **Esau, A.**: Elektrotechnische Ztschr., **47**, 321, 1926. (9.) **Fiandaca, S.**: Arch. Phys. Ther., *X*-ray and Radium, **18**, 79, 1937. (10.) **Haas, M.**, and **Lob, A.**: Deutsch. Ztschr. f. Chir., **243**, 318, 1934. (11.) **Haase, W.**, and **Schliephake, E.**: Strahlentherapie, **40**, 133, 1931. (12.) **Hasche, E.**: München. med. Wchnschr., **85**, 1033, 1938. (13.) Cited by de Cholnoky,[5a] p. 194. (14.) **Izar**: Atti. acc. med. Lombarda, maggio, 1935 (cited by Cignolini[1]). (15.) **Koslin**: Cited by Liebesny.[19a] (16.) **Kowarschik, J.**: (*a*) Kurzwellentherapie, Vienna, Julius Springer, p. 129, 1936; (*b*) Ibid., p. 106. (17.) **Krusen, F. H.**: J. Am. Med. Assn., **110**, 1280, 1937. (18.) **Küttner-Dittrich**: Cited by Liebesny.[19a] (19.) **Liebesny, P.**: (*a*) Kurz- und Ultrakurzwellen, Berlin, Urban und Schwarzenberg, p. 104, 1935. (*b*) Ibid., p. 144; (*c*) Ibid., p. 186. (*d*) Die Kurzwellentherapie in möglichst athermischer Dosierung, Internationaler Kongress für Kurzwellen, Wien, 1937, Vienna, Moritz Perles, p. 175, 1937. (20.) **Liebesny, P., Wertheim, H.**, and **Scholz, H.**: Klin. Wchnschr., **12**, 141, 1933. (21.) **Maes, U.**: Ann. Surg., **106**, 1, 1937. (22.) **Mittelmann, E.**: Arch. Phys. Ther. and Radium, **18**, 63, 1937. (23.) **Pflomm, E.**: Arch. klin. Chir., **166**, 251, 1931. (24.) **Raab, E.**, and **Hofmann, J.**: Deutsch. med. Wchnschr., **17**, 699, 1936. (25.) **Rausch, Z.**: Fortschr. d. Ther., **10**, 394, 1934. (26.) **Ruete, A. E.**: Ueber die Wirkung von Kurzwellen auf Bacterium coli und pathogene Pilze, sowie, über ihre Brauchbarkeit in der Behandlung dermatologischer Erkrankungen, Internationaler Kongress für Kurzwellen, Wien, 1937, Vienna, M. Perles, p. 266, 1937. (27.) **Schereschewsky, J. W.**: (*a*) Personal communication; (*b*) Pub. Health Rep., **41**, 1939, 1926; **43**, 927, 1928. (28.) **Schliephake, E.**: (*a*) Klin. Wchnschr., **7**, 1600, 1928; Verhandl. d. deutsch. Gesellsch. inn. Med., **40**, 307, 1928; (*b*) Kurzwellentherapie, Jena, Gustav Fischer, p. 116, 1932; (*c*) Ibid., Berlin, Julius Springer, p. 132, 1932; (*d*) Die klinische Verwendung der elektrischen Ultrakurzwellen, Klemperer's Klinische Fortbildung, 1 Jahresband, Berlin, Urban und Schwarzenberg, p. 478, 1933. (*e*) Kurzwellentherapie, Jena, Gustav Fischer, p. 136, 1936; (*f*) Ibid., Berlin, Julius Springer, p. 152; (*g*) Wirkungsweise und Indikationes der Kurzwellen, Internationaler Kongress für Kurzwellen, Wien, 1937, Vienna, M. Perles, p. 233, 1937. (29.) **Weiss, H., Pick, J.**, and **Tomberg, V.**: Arch. Phys. Ther. and Radium, **19**, 79, 1938. (30.) **Weissenberg, E.**: Ibid., **18**, 551, 1937.

THE SIGNIFICANCE OF SMALL AND ABSENT INITIAL POSITIVE DEFLECTIONS IN THE CHEST LEAD.

By Joseph B. Vander Veer, M.D.,

INSTRUCTOR IN MEDICINE, UNIVERSITY OF PENNSYLVANIA; PHYSICIAN TO THE PENNSYLVANIA HOSPITAL, PHILADELPHIA, PA.,

AND

Joseph C. Edwards, M.D.,*

ASSOCIATE IN MEDICINE, WASHINGTON UNIVERSITY MEDICAL SCHOOL; ASSISTANT PHYSICIAN, BARNES HOSPITAL, ST. LOUIS, MO.

(From the Heart Station and the Medical Services of the Pennsylvania Hospital.)

In the past few years there have been numerous articles dealing with the precordial leads of the electrocardiogram. These studies, both clinical and experimental, have been carried out with a variety

* Morris W. Stroud, Jr., Fellow in Cardiology, Pennsylvania Hospital, 1937–38.

of techniques. Recently[1] an attempt has been made to standardize the precordial leads used in clinical electrocardiography, especially for routine work where a single precordial lead is used. This is a distinct step forward and will eliminate much confusion which has existed in the past. The whole matter is not finally settled and the recommendations for standardization were not intended to discourage further investigative work.

The normals have been quite well defined and certain facts have been established in relation to the various components of the precordial electrocardiogram. The following changes have been frequently observed and certain clinical associations have been noted (the precordial electrode being placed on the area of the cardiac apex beat and the other electrode on the left infrascapular area or left leg).

1. The initial positive deflection of the *QRS* complex (*Q* wave of old technique, *R* wave of new technique) is practically never absent in normal hearts.[5,7,9a,10]

2. Absence of the initial positive deflection is most often seen following a recent or old myocardial infarction at the apex of the left ventricle anteriorly.[2,11a,b,12] A few other conditions, nearly all organic heart disease, may cause absence of this deflection.

3. A small initial positive deflection (less than 2 mm.) is frequently seen in various types of organic heart disease.[6,8] It is a less valuable sign than an absent initial deflection and probably is seen occasionally in normal hearts. In many patients the height of the initial deflection varies greatly with the position of the precordial electrode. In these cases the point of application of the electrode may be responsible for an abnormally small initial deflection.[4]

4. Changes in the *T* waves of the precordial lead are usually associated with changes in the same wave in Lead I. Occasionally an abnormal *T* wave in the chest lead is the only significant change present in the electrocardiogram.[3] A normal *T* wave is not infrequent in the precordial lead with inversion of the *T* waves in one or more of the limb leads, especially Leads II and III. The position of the precordial electrode may alter greatly the size and contour of the *T* wave in this lead.[9b]

Important changes in the *QRS* complex of the precordial lead (absent or small initial positive deflection) may be present with little or no change in the *QRS* or *T* waves of the limb leads. There is considerable evidence to the effect that these changes, when present, tend to be more permanent in nature than *T* wave abnormalities. For this reason they may remain as residual findings (especially after myocardial infarction), when the *T*-wave alterations have returned to normal. It should be remembered that severe heart damage may be present with little or no alteration in the precordial lead and that the significance of abnormal findings in the limb leads is not altered by a normal Lead IV.

It seemed worth while to us to analyze the electrocardiograms in our files in which an absent or small initial positive deflection was noted in the precordial lead. The correlation of these changes with abnormalities in the limb leads and *T*-wave changes in both the precordial and limb leads seemed important. The clinical histories of these selected cases were then studied in order to determine the presence or absence of heart disease and the etiologic and anatomic diagnoses.

For the past 3 years at the Pennsylvania Hospital a precordial lead has been recorded routinely on all adults on whom an electrocardiogram was taken. Lead V of Wolferth and Wood[11b] (R.A. electrode on the cardiac apex and L.L. electrode on the left leg) was used during most of this period. For 2 years prior to this, Leads IV, V and VI (Wolferth and Wood) were taken in cases in which coronary disease was suspected. Several months prior to the completion of this study, the routine precordial lead was changed to Lead IV R (L.A. electrode on apex and R.A. electrode on right arm). This last lead is one of those recently recommended for routine use by the American Heart Association.[1] It has the advantage of being comparable to the limb leads and is essentially the inverted mirror image of the old chest lead. The records from this study were taken from approximately 10,000 consecutive records during the above period. There were records from 102 patients with an absent initial positive deflection in the precordial lead (*Q* wave of the old technique and *R* wave of new), and from 46 patients with a small initial positive deflection (less than 2 mm.) in the precordial lead.

Technique. The precordial electrode used during most of the study was a German silver electrode measuring approximately 4 by 6 cm. This was changed to a circular electrode 3 cm. in diameter during the last few months of the observations. The electrode was placed as nearly as possible on the cardiac apex as determined by palpation and percussion. In patients with very large hearts the electrode was placed somewhat medial to the apex and was seldom placed outside the anterior axillary line. (Observations on a number of patients with greatly enlarged hearts led us to believe that it was unwise to place the precordial electrode on or "just outside" the apex beat in these cases, as has been recommended for routine use. The results of a special study on a total of 67 patients with normal and abnormal hearts, using six precordial areas for the exploring electrode and the right arm, left arm and left leg, respectively, as the indifferent electrode, will be reported in a separate paper.[4]) The indifferent electrode was placed on the left leg during most of this study. The use of the right arm for application of the indifferent electrode (Lead IV R) was found to be as satisfactory and as a matter of convenience was used in the latter part of the study.

Analysis of Cases With an Absent Initial Positive Deflection. There were 102 patients in this series with an absent initial positive deflection. A total of 260 records were taken on these patients. Fifty-three of these cases had 2 or more records. In 49 cases only

1 record was obtained. Of the group with only 1 record, 23 had the electrocardiographic pattern of a recent myocardial infarction at the time the examination was made. A total of 68 patients (67%) had clinical and electrocardiographic evidence of a recent or an old myocardial infarction (Figs. 1 and 2). There were 45 males and 23 females. In all of these patients the location of the infarct as determined by the electrocardiographic pattern was anterior, or both anterior and posterior. Two of the patients had had an old anterior and a more recent posterior infarction. In 1 patient the acute infarct was probably both anterior and posterior, mainly the former, as determined by the electrocardiographic pattern.[11c] In all of the fatal cases in which postmortem examination was done, the location of the infarct or scar was found to correspond to the area predicted by the electrocardiogram. The youngest of these patients was a male of 39 years, and 58 patients (85%) were between the ages of 40 and 70 years. In 5 of these patients (7.3%) with coronary occlusion, the only significant change in the electrocardiogram, at the time of this analysis, was in the precordial lead. In 11 patients of this group (16%), the main electrocardiographic change was in the precordial lead. Some of these were cases in which the occlusion had occurred several months or years previously, and in many, other abnormalities may have been present in the limb leads during the acute stages. A correlation of the *T*-wave changes in Leads I and IV is of interest and is shown in Table 1. In only 2 cases were the *T* waves normal in both of these leads. In 55 cases the *T* waves were abnormal in both of these leads.*

There were 34 patients in the group with absent initial positive deflection in the chest lead (33%) in which there was no clinical history or findings suggestive of myocardial infarction. Nineteen of these patients were males and 15 females. The youngest patient was a woman of 38 years, and 28 of the total (82%) were between the ages of 40 and 70 years. In 5 of this group (14%) the absent initial positive deflection was the only abnormality in the electrocardiogram. In 10 other patients (29%) the main change in the electrocardiogram was the absent *Q* or *R* wave. This was nearly double the per cent of cases in which the entire or main abnormality was seen in the precordial lead in the patients with myocardial infarction. A comparison of the abnormal *T* waves in Leads I and IV is of interest also in the patients without infarction. In this group the *T* waves were normal in both of these leads in 11 cases. They were both abnormal in only 14 of the 34 cases, and the *T* waves were much more frequently abnormal in the first lead alone than in the precordial lead alone (Table 1).

The etiologic diagnoses were determined from the clinical records and from postmortem studies where such information was available.

* A *T* wave was considered abnormal when it was inverted or diphasic or flat, if digitalis had not been given.

Of the 102 cases, 95 (93%) had arteriosclerotic (coronary) heart disease; 50 of these also had hypertension, and 3 hypertension alone. Six patients had luetic aortitis. This was confirmed at postmortem in 4 of these 6 cases, and in 2 cases there was also myocardial infarction due to the closure of the coronary orifices. Two of the cases with aortitis were also included in the arteriosclerotic group.

TABLE 1.—A COMPARISON OF THE *T*-WAVE CHANGES IN LEADS I AND IV IN 102 CASES WITH AN ABSENT INITIAL POSITIVE DEFLECTION IN THE PRECORDIAL LEAD.

	T 1 and *T* 4 abnormal.	T 1 abnormal T 4 normal.	*T* 1 normal *T* 4 abnormal.	*T* 1 and *T* 4 normal.
68 cases with myocardial infarction	55 (81%)	6 (9%)	5 (7%)	2 (3%)
34 cases with no infarction . .	14 (41%)	10 (29%)	0 0	10 (30%)

Analysis of Cases With a Small Initial Positive Deflection. There were 46 patients with a small initial positive deflection in the precordial lead (a *Q* or *R* wave of 2 mm. or less was regarded as a "small" initial positive deflection) (Fig. 3, *B*). A total of 85 records were obtained on these patients. In 26 patients 2 or more records were taken. In 20 patients only one electrocardiogram was available. In those cases with several records, it was not uncommon to find a record with the initial positive deflection of normal amplitude. In most of these cases it was felt the variation was the result of a difference in the position of the precordial electrode rather than any improvement or change in the cardiac status (Fig. 3, *A*). Further evidence that this was a technical phenomenon was suggested by the fact that some cases with several records would have a normal precordial lead interposed between 2 records with a small initial positive deflection or *vice versa*. Such cases were not included in this series, but were a further stimulus to investigation of the size of the initial positive deflection, using multiple precordial leads in normal and diseased hearts. We are in agreement with Master *et al.*[8] that a correlation of the height of the initial deflection with the height of the second wave (*S* wave of the new technique, *R* wave of the old technique) is desirable. If the second deflection is less than 10 mm. in amplitude the small initial positive deflection is often of no significance and will often be found within the limits of normal on subsequent records with greater amplitude. Cases of this type were not included in this study.

There were 28 females and 18 males in the group with small initial positive deflection. This was in contrast to the absent initial positive deflection group where the males were nearly twice as numerous as the females. Twelve of this group of 46 were below 40 years, while only 2 of the other much larger group were below this age. There were 28 patients (60%) between the ages of 40 and 70 years.

The etiologic diagnoses, as determined largely from clinical study, in the group of cases with *small* initial positive deflections in the precordial lead were somewhat comparable to the larger group in which the initial deflection was *absent*. In 28 patients (61%), a diagnosis of arteriosclerotic (coronary) heart disease was made; 15 of these also had hypertension; 6 (13% of the total) had probably had a previous myocardial infarction (anterior) as determined by the clinical history or previous electrocardiograms, or both (Fig. 4, *B*). One patient had hypertension with probably no significant coronary disease; 7 patients had rheumatic heart disease and in 5 instances the etiologic agent was syphilis. Four cases had normal hearts clinically, although 1 was abnormally placed because of dorsal scoliosis. This is in contrast to the larger group of cases with an absent initial positive deflection in which cardiovascular disease was present in every case (Table 2).

TABLE 2.—A COMPARISON OF THE *T*-WAVE CHANGES IN LEADS I AND IV IN 46 CASES WITH A SMALL INITIAL POSITIVE DEFLECTION IN THE PRECORDIAL LEAD, AND THE ETIOLOGIC FACTORS CONCERNED IN EACH GROUP.

Etiology.	*T* 1 and *T* 4 abnormal.	*T* 1 abnormal *T* 4 normal.	*T* 1 normal *T* 4 abnormal.	*T* 1 and *T* 4 normal
Arteriosclerosis	5	1	0	7
Arter. and hyper.	4	6	1	4
Hypertension	0	0	0	1
Rheumatic fever	3	1	1	2
Syphilis	3	0	1	1
Hyperthyroidism	1	0	0	0
Normal	0	0	0	4
Total cases	16 (35%)	8 (17%)	3 (7%)	19 (41%)

In 12 patients of this group (26%), the *small* initial positive deflection was the only electrocardiographic abnormality present and in 7 (15%) it was the main abnormality. A comparison of the *T*-wave changes in Leads I and IV, as was done in the absent initial positive deflection cases, is of interest. In 19 cases (41%), the *T* waves were normal in both leads. In 16 (35%), the *T* waves were abnormal in both leads. *T* 1 was abnormal and *T* 4 normal in 8 cases (17%), and an abnormal *T* 4 with a normal *T* 1 was found in 3 cases (7%). Table 2 correlates the *T*-wave changes with the clinical diagnoses.

Postmortem Studies. In the group of 102 patients with *absent* initial positive deflections there were 30 deaths and autopsies were done in 17 instances. In 9 cases death seemed attributable to a recent myocardial infarction. This was located anteriorly at or near the apex (in some instances involving the interventricular septum) in 7. In 1 instance, there was an old localized scar at the apex of the left ventricle with a recent infarct posteriorly at the base of the same ventricle (Fig. 4, *A*). In the other case, there was a similar anterior scar and a recent infarct on the midlateral aspect

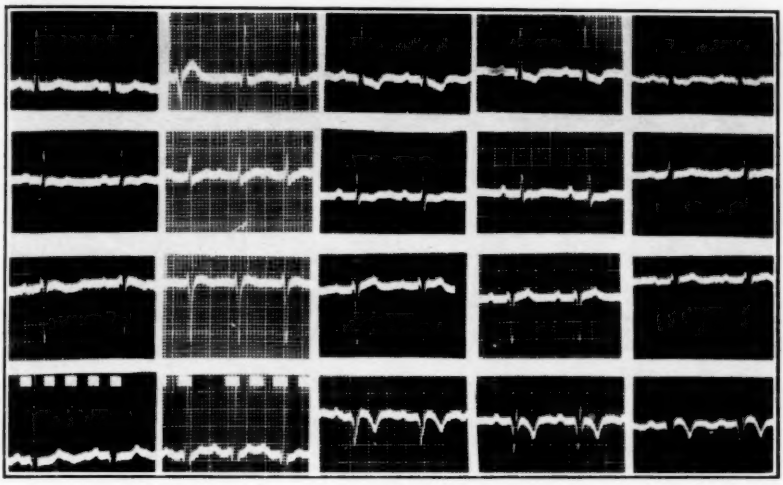

FIG. 1.—Case J. Z., Italian male, aged 68. Arteriosclerotic heart disease with moderate hypertension; Wassermann test positive. Dyspnea on exertion for 3 years, but no precordial pain. Mild cardiac failure present at the time of the record of Sept. 18. Note the relatively slight abnormalities present on this date. Coronary occlusion, Dec. 20. On Dec. 22, the electrocardiogram shows but slight change from the previous record and digitalis had been given for some time prior to this. The tracing of Dec. 29 shows a striking change in the *T* waves in Lead I and the presence of a deep *Q* wave in the precordial lead (IV R) with inversion of the *T* waves in this lead. Feb. 18, the *T* waves in Lead I are beginning to return to normal but the *QRS* and *T* wave changes persist in the precordial lead.

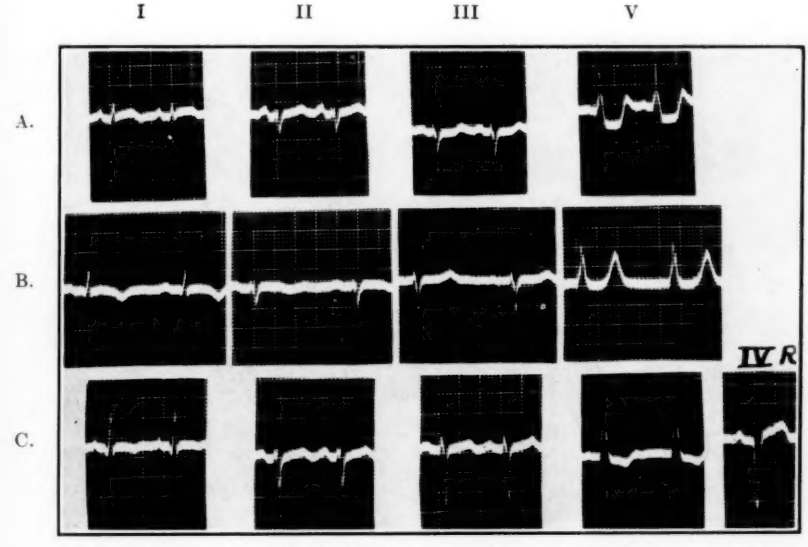

FIG. 2.—Case C. S., colored male laborer, aged 53. Arteriosclerotic heart disease with cardiac enlargement. Typical symptoms of coronary occlusion, Nov. 3, 1936. Gradual improvement but still great limitation of the cardiac reserve to date. *A*. Nov. 5, 1936. Electrocardiographic pattern of acute anterior myocardial infarction. Chest lead (Lead V of Wolferth and Wood) shows an absence of the initial positive deflection (*Q* wave in this instance) and diphasic *T* waves. *B*. Dec. 4, 1936. Further *T*-wave changes and persistence of the absent initial deflection in the chest lead. *C*. July 12, 1938. This record shows much less inversion of the *T* waves in Lead I and return of the *T* waves to normal in the precordial lead. The new chest Lead IV R (apex and right arm) is shown for comparison. Note that the absent initial positive deflection (*Q* wave of the old and *R* wave of the new techniques) has not reappeared in the precordial leads.

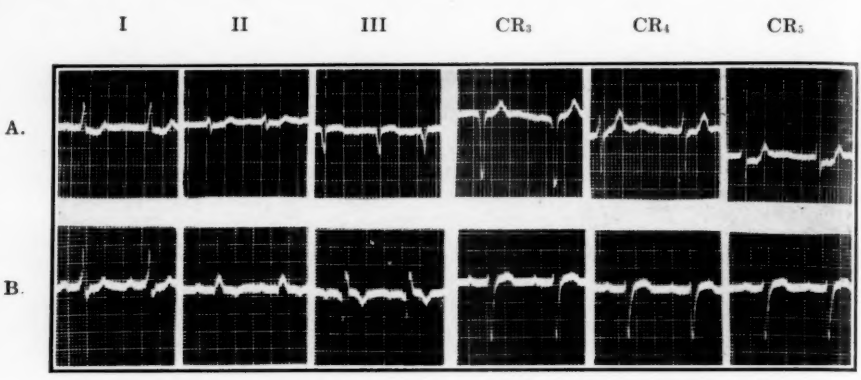

FIG. 3.—*A*. Case M. A., white housewife, aged 67. Old rheumatic heart disease with mitral stenosis, arteriosclerosis and hypertension. Marked cardiac enlargement and auricular fibrillation. Digitalized. The limb leads show auricular fibrillation, diphasic *T* waves and left axis deviation. Three precordial leads are shown, using the right arm as the indifferent electrode and the precordial electrode on the parasternal line, *CR* 3; midclavicular line, *CR* 4; and the anterior axillary line, *CR* 5. Note the marked variation in the ventricular complexes in the different positions. The initial positive deflection (*R* wave) is absent in the medial position, within limits of normal at the midclavicular line and large at the lateral position. *B*. Case M. C., housewife, aged 59. Arteriosclerotic heart disease with cardiac enlargement. History suggestive of old and recent myocardial infarctions. The limb leads show the *QRS* and *T*-wave changes in keeping with a fairly recent posterior infarction. A slight delay in the *A–V* conduction time also is present. The chest leads show little change in the *QRS* complexes in the different positions. The small *R* wave is probably the residual finding of an old anterior myocardial infarction in this instance.

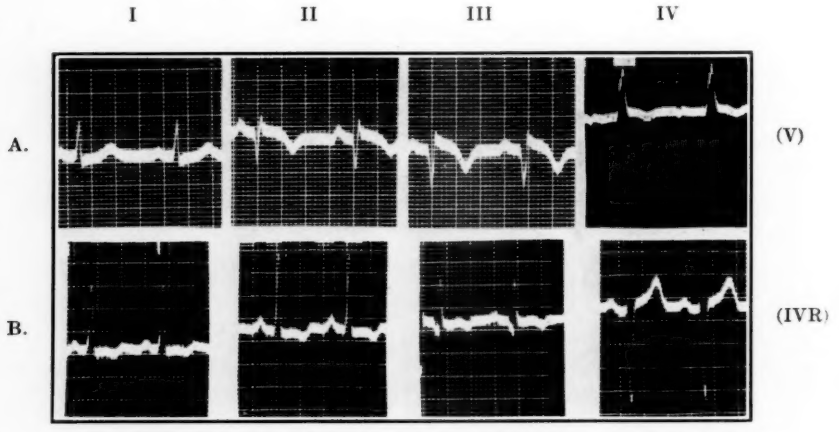

FIG. 4.—*A*. Case H. R., white male, aged 61. Atypical but severe attack of coronary occlusion followed by the clinical course of myocardial infarction. Similar attack 6 years before with good recovery after a few days in bed. Sudden death on the fifth day after the onset. Note the pattern of acute posterior infarction in the limb leads (third day). The chest lead (Lead V of Wolferth and Wood), taken the following day, shows an absence of the normal initial positive deflection (*Q* wave) with normal *T* wave. A delay in the *P–R* interval is also present. The diagnosis of recent posterior infarction seemed evident and, on the basis of the chest lead findings (absent initial positive deflection) and the history, a diagnosis of old anterior infarction was made. Postmortem examination proved both to be correct. *B*. Case E. P., colored housewife, aged 42. Syphilitic aortitis and aortic insufficiency with marked cardiac enlargement. Blood pressure, 190/60. Dyspnea on exertion and some precordial pain. Definite *T* wave changes are present in the limb leads. The chest lead (IV R—new technique) shows a normal *T* wave but a small to absent initial positive deflection (*R* wave). This finding alone is strong evidence that myocardial changes are present.

of the left ventricle with rupture through the ventricular wall and hemopericardium. In 4 of the autopsies a localized scar was found on the anterior surface of the left ventricle near the apex, the result of an old coronary occlusion. The area of infarction or scarring was apparently secondary to coronary thrombosis in all but 1 case where closure of the orifice of the left coronary artery resulted from syphilitic aortitis. Two cases showed coronary sclerosis with diffuse scarring of the left ventricle but no localized lesion. One of these patients died as the result of cardiac failure and the other from cerebral hemorrhage. In the latter case, signs and symptoms of cardiac insufficiency had been present some months before death. Syphilis was the etiologic agent in the 2 final cases. Luetic aortitis with involvement of the aortic valve were present in both instances with cardiac hypertrophy, dilatation and myocardial fibrosis. Death had resulted from cardiac failure. Hypertension had been present in 12 of the 17 cases during life; being present in 8 of the cases with recent or old infarction, in the 2 coronary sclerosis cases without infarction, and in both of the cases with syphilitic aortitis and aortic insufficiency.

There were 8 deaths in the group of 46 patients with a small initial positive deflection in the precordial lead. Postmortem examinations were done in 4 of the cases. In all of these, organic heart disease was found, though in 2 instances death resulted from other causes. The anatomic diagnoses were as follows: Luetic aortitis with cardiac enlargement and myocardial fibrosis; rheumatic heart disease with mitral stenosis and aortic insufficiency and extreme hypertrophy and dilatation (heart weighed 720 gm.); arteriosclerotic heart disease with cardiac enlargement (history of hypertension). The final case showed cardiac enlargement (520 gm.) with diffuse degenerative changes in the myocardium but little gross coronary disease. Clinically, auricular fibrillation was present and death resulted from congestive heart failure in this case.

Discussion. It has been recognized for some time that the initial positive deflection of the precordial lead (*R* wave of new technique and *Q* wave of old technique) is frequently absent following infarction of the anterior wall of the left ventricle. The same is not true of infarction of the lateral and posterior walls of this ventricle. A few cases have been reported in which this wave was absent and apparently no cardiac disease was present. In our series of 102 cases with this finding, however, organic cardiovascular disease was present in every case. The findings compare closely to those of Master *et al.*[8] in this group, approximately two-thirds of the cases in both series having recent or previous myocardial infarction. The electrocardiographic pattern of all of our cases suggested the area of infarction or scar was anterior or both anterior and posterior. This finding was confirmed in all of the postmortem examinations. We found no instances of typical posterior infarction alone in which

the initial positive deflection was absent. That this wave may be absent in conditions other than myocardial infarction is obvious from analysis of our records and from cases reported by other authors. Our series of cases with this sign were limited nearly entirely to arteriosclerotic (coronary) heart disease excepting a few patients with luetic aortitis. Most of these latter were complicated by hypertension or coronary orifice involvement. The exact cause of absence of the initial positive deflection has not been finally determined and it is probable that several different factors may be involved. All writers agree that anterior infarction is the most frequent cause. Master *et al.*[8] believe cardiac enlargement or rotation may often be the factor and that even capillary damage (?) (acute nephritis) may occasionally cause absence of this wave. In our cases in which myocardial infarction was not present, myocardial damage secondary to coronary or aortic disease was the usual apparent cause of absence of the initial positive deflection. Cardiac enlargement may also have been a factor in some of these cases. That severe myocardial damage, cardiac enlargement, and even anterior infarction may be present without abnormal changes in the precordial lead is well known and tends to complicate the problem. This fact, however, must be recognized and borne in mind when interpreting electrocardiograms with abnormal findings in the limb leads accompanied by a normal precordial lead.

When the initial positive deflection of the precordial lead is completely absent (if the technique of taking the lead has been correct), it probably rarely returns to normal. In 1 of our cases (proved at postmortem), this wave was still absent 6 years after the occurrence of an anterior infarct (Fig. 4, *A*). In general, the *QRS* abnormalities in the precordial lead seem to be less susceptible to change (return to normal) than do the *T*-wave changes in this lead.

The presence of a *small* initial positive deflection (2 mm. or less) in the precordial lead is often of similar significance but we believe a definitely less important sign than an absent deflection. Our studies confirm the fact that an absent initial positive deflection as the result of an anterior infarct may be followed by a small deflection after a period of a few months. Master *et al.*[8] found that a small initial deflection was almost as frequent in posterior as in anterior wall infarcts. Analysis of our cases did not bear out this finding, though our series was considerably smaller. Our findings do confirm the statement of these authors that a small initial positive deflection is of little significance unless the second deflection of the *QRS* complex (*S* wave of new and *R* wave of old techniques) is 10 mm. or more in amplitude. The position of the precordial electrode is an important factor in the size of the initial deflection in many cases, and probably accounts for most of the variations in the size of the initial deflection seen in serial records. With this electrode placed near the sternum there is a tendency for a small or

even absent initial positive deflection in some cases which have a normal complex with the electrode placed on the apex area (Fig. 3, *A*). When a small initial positive deflection is the only abnormal electrocardiographic finding, it would seem desirable that the record be checked by another examination, with careful attention being paid to the position of the precordial electrode. This is especially true if the precordial electrode is placed on the chest routinely by a technician. As we have stated in a separate publication,[4] we believe that the most desirable single routine position for applying the precordial electrode on the chest is at the apex area, in normal and slightly enlarged hearts, and slightly medial to the apex in patients with large hearts.

Correlation of the *T*-wave findings in Leads I and IV with the absent and small initial positive deflections in the precordial lead (IV) (Tables 1 and 2) shows a much higher per cent of abnormal *T* waves in the absent initial deflection group than in those with small initial deflections. In the group of cases with absent initial positive deflections and a definite diagnosis of old or recent myocardial infarction, the *T* waves were abnormal in 97% of the (68) cases in one or both of these leads. They were abnormal in both of these leads in 81% of the cases. In the small initial positive deflection group the *T* waves were abnormal in 59% in either lead and in only 35% of the cases in both Leads I and IV. This would seem further evidence proving the greater significance of the absent as compared to the small initial positive deflection. If the absent or small initial positive deflection is accompanied by abnormal *T* waves in any lead, especially Leads I or IV, the chances are greater that cardiac damage is present. The presence of these abnormalities even alone, however, is strong evidence of cardiac involvement.

Summary. 1. The electrocardiograms and clinical histories of 102 patients with an absent initial positive deflection in the precordial lead (*R* wave of new and *Q* wave of the old technique), and 46 patients with a small initial positive deflection (2 mm. or less) have been reviewed.

2. The most frequent cause of an absent initial positive deflection (67%) was a previous anterior myocardial infarction. In the other patients with this abnormality, organic cardiovascular disease was present in all instances, usually arteriosclerotic or luetic heart disease, with or without hypertension. Cardiac enlargement and myocardial damage from other causes may also be factors causing disappearance of this wave.

3. When the initial positive deflection is absent in the precordial lead, the *QRS* complex rarely returns to normal. An *absent* initial deflection may be followed by a *small* initial deflection in some cases of myocardial infarction and occasionally either of these signs may be the only residual electrocardiographic finding in this condition.

4. A small initial positive deflection in the precordial lead (2 mm.

or less) is a less important sign than an absent one. It is occasionally seen in normal hearts but usually is of similar significance to an absent initial positive deflection.

5. The importance of proper application of the precordial electrode is emphasized, especially in relation to the size of the initial positive deflection of the *QRS* complex.

6. A correlation between the *T*-wave findings in the precordial lead and Lead I with the absent and small initial positive deflections shows a larger per cent of abnormal *T* waves in the absent deflection group. The presence of an abnormal *T* wave in one or both of these leads with a small or absent deflection in the precordial lead makes the latter sign of greater significance. In general, *T*-wave abnormalities are less permanent than the *QRS* abnormalities in the precordial lead.

We are indebted to Dr. D. W. Dominick for assistance in studying some of the cases.

REFERENCES.

(1.) American Heart Assn. and Cardiac Soc. of Great Britain and Ireland, Joint Recommendations of, Am. Heart J., **15**, 107, 235, 1938. (2.) **Bellet, S.**, and **Johnston, C. G.**: J. Clin. Invest., **13**, 725, 1934. (3.) **Edeiken, J., Wolferth, C. C.**, and **Wood, F. C.**: Am. Heart J., **12**, 666, 1936. (4.) **Edwards, J. C.**, and **Vander Veer, J. B.**: Ibid., **16**, 431, 1938. (5.) **Katz, L. N.**, and **Kissen, M.**: Ibid., **8**, 595, 1933. (6.) **Levine, E. D.**, and **Levine, S. A.**: Am. J. Med. Sci., **191**, 98, 1936. (7.) **Master, A. M.**: Am. Heart J., **9**, 511, 1934. (8.) **Master, A. M., Dack, S., Kalter, H. H.**, and **Jaffe, H. L.**: Ibid., **14**, 197, 1937. (9.) **Roth, I. R.**: (*a*) Ibid., **10**, 798, 1935; (*b*) Ibid., **14**, 155, 1937. (10.) **Shipley, R. A.**, and **Hallaran, W. R.**: Ibid., **11**, 325, 1936. (11.) **Wolferth, C. C.**, and **Wood, F. C.**: (*a*) Med. Clin. North America, **16**, 161, 1932; (*b*) Am. J. Med. Sci., **183**, 30, 1932; (*c*) Arch. Int. Med., **56**, 77, 1935. (12.) **Wood, F. C., Bellet, S., McMillan, T. M.**, and **Wolferth, C. C.**: Arch. Int. Med., **52**, 752, 1933.

ALLERGY AS A FACTOR IN THE DEVELOPMENT OF REACTIONS TO ANTI-RABIC TREATMENT.

By Harold M. Horack, M.D.,*

MEDICAL HOUSE OFFICER, PETER BENT BRIGHAM HOSPITAL, BOSTON, MASS.

(From the Department of Medicine, Duke University School of Medicine, Durham, N. C.)

Rabies as a disease entity is one of the oldest known. Records of its existence date from the time of Aristotle. Celsus, in the first century A. D., was the first to give a detailed description of human rabies, and to state that the disease was the result of a bite by a rabid animal. Centuries passed but nothing of importance was added to our knowledge of the disease until 1879 when Galatier and Pasteur proved that rabies was an infectious disease. The famous experimental work of Pasteur followed, and in 1885 the Pasteur treatment was introduced as a prophylactic measure in the treatment of rabies.

* Formerly of Durham, N C.

However, between the years 1885 and 1898 doubts developed concerning the safety of this preventive measure because of the occasional occurrence of paralyses during or after the course of treatment. These paralyses were believed to be due to the street virus or the result of its modification in the course of treatment. In 1898, Tonin[20] opposed this view by recording a case in which paralysis developed in spite of the fact that the animal suspected of having rabies never developed the disease. He suggested therefore, that the paralysis was due to the treatment *per se*.

Incidence of Paralytic Accidents. Considering the frequency with which the Pasteur treatment is administered, the incidence of accidents resulting from such treatment is fortunately of rare occurrence. Nevertheless, the fact that they do occur and that they may be extremely severe, constitutes a serious objection to the treatment unless positively indicated. At the First Rabies Conference of The League of Nations[14] a study of the accidents resulting from the Pasteur treatment (and its modifications) revealed the development of 329 accidents in 1,164,264 persons treated. Since then McKendrick[13a,b] has reported an additional 33 cases in 170,904 persons who were treated between 1926 and 1930. From the combined reports, the incidence of paralytic accidents appears to be 1 case per 3668 treatments, (0.027%).

The North Carolina State Board of Health has distributed approximately 24,000 courses of anti-rabic treatment (Calmette modification) and has endeavored to record all severe accidents attributable to the treatment. At the present time 20 such cases have been reported. The incidence of accidents with the treatment has been 1 per 1200 (0.083%). The mortality rate of those suffering untoward reactions has been, in this State, approximately 10% as compared with a mortality rate of 16% recorded by Remlinger and others.[14] In spite of the relatively small group of patients receiving treatment through the State Board of Health, these percentages are of significance in that physicians in this State, using the State-prepared vaccine, have without hesitation revealed their misfortune to the Health Laboratory. This is in contrast to some Pasteur Institutes which, for statistical or other reasons, have been reluctant to reveal their own unfortunate results.

Classification of Reactions. In administering the Pasteur treatment one should be mindful of the various types of reactions that may be encountered. For practical purposes these can be divided into 6 groups:

GROUP 1. The prompt development of a *generalized urticarial rash* which responds to the use of adrenalin, and which usually develops in those who have been sensitized by the previous injection of nervous tissue. These cases are relatively infrequent and are without mortality.

GROUP 2. *Delayed reactions of the tuberculin type* occurring at the

site of injection and characterized by local redness, induration, tenderness, and itching. They are the most common type of local reaction encountered. They occur early during the treatment, but are not of serious import.

GROUP 3. *Reaction of the type described as Group 2, but more severe,* and frequently associated with constitutional reactions consisting of headache, low-grade fever, lymph node enlargement, nausea, and general feeling of malaise. Subsequent injections are apt to cause a flare-up at the previous injection sites. It is in these patients that one should observe closely for signs of the development of paralysis, and judge their treatment accordingly. These cases are less common than those described in Group 2.

GROUP 4. *The simple peripheral neuritic form,* following a short febrile attack and commonly involving the facial nerve.

GROUP 5. *A dorso-lumbar myelitis,* usually occurring during the second or third week of treatment, and characterized by the gradual onset of fever, weakness, numbness, tingling of the lower extremities, sphincter disturbances, and terminating in paralysis of the extremities. The local reactions are of the more severe type described under Group 3. The mortality rate is low.

GROUP 6. *Paralysis of the Landry type,* which is sudden in onset and is associated with high fever, nausea, insomnia, vomiting, headache, girdle pains, retention of urine, and an ascending paralysis. About one-third of these patients die of bulbar paralysis. Local reactions are those of Group 3.

To the types of reactions mentioned above may be added the case which is reported below. A thorough review of the available literature reveals no comparable case. The majority of the accidents previously reported are typically encephalomyelitic, whereas this case manifested an encephalitis without any paralysis.

Case Report. *Duke Hosp. No. 1921. A white male of 22 bitten by a cat suspected of having rabies: anti-rabic treatment: development of encephalitis after twelfth treatment: complete recovery.* H. M. H., a 22-year-old medical student, while working with a cat, was bitten and scratched. The animal had been exposed to rabies and treatment was advised. He received his first injection of the Calmette modification of the Pasteur treatment on the sixth day following exposure. The injections were given subcutaneously, 4 injections in the back alternating with 4 in the abdomen. Following the fifth injection the axillary lymph nodes enlarged and became quite tender. Following the sixth injection, sites of previous injections became moderately red, tender, and indurated. There was a generalized pruritus, but no rash developed. These signs and symptoms were progressive and at the time of the twelfth injection the patient complained of headache, backache, restlessness, fever, loss of appetite, and nausea. He was given the twelfth treatment and instructed to remain in bed. The following day, because of an increase in the severity of the symptoms, he was admitted to the hospital.

Upon admittance the patient appeared acutely ill. He was very drowsy, had great difficulty in concentrating, and answered questions slowly. He complained of a severe headache and of being nauseated. His features were expressionless. The temperature was 39.5° C., pulse 100, respirations 20.

The general physical and neurologic examinations were negative except for marked induration at the injection sites, generalized glandular enlargement, difficulty on convergence of the eyes, slight papilledema, and a slightly stiff neck. The patient's history was interesting in that his paternal grandfather had asthma, his father and brother hay fever, and his mother exhibited marked idiosyncrasies to various foodstuffs. The patient was moderately sensitive to horse serum, was subject to sinusitis, and a Brucella skin test (given 1 year earlier) gave a 4+ reaction with necrosis and sloughing of the tissues of the forearm. His habits were regular and he had previously enjoyed excellent health.

Following admission to the hospital his condition became progressively more acute. The temperature remained elevated (38.5° to 39.5° C.), the headache became more severe, and the anorexia and sense of nausea persisted. He vomited occasionally, and he developed a rather marked bradycardia (48 to 52). The lethargy increased and by the third hospital day he was quite stuporous. On lumbar puncture, the fluid was found to be under a pressure of 250 mm. of spinal fluid. Examination of the fluid revealed 164 mononuclears and 2 neutrophils per c.mm. of spinal fluid; Pandy, 1+; the colloidal mastic test gave a 3332100 curve; colloidal gold test, benzidene reaction and bacterial cultures were negative. The puncture caused a transitory improvement in his condition. A second lumbar puncture was done on the fifth hospital day and the fluid was found to be under a 270 mm. pressure, but there was no appreciable change in the character of the fluid. This procedure was again followed by improvement in his general condition. A third lumbar puncture was done 6 hours later. On the sixth hospital day he appeared markedly improved. He was alert, rational, conversational, and relatively comfortable. The pulse rate was 70 and the temperature 38° C. Rabbits and guinea pigs inoculated with the spinal fluid remained healthy. Blood and spinal fluid Wassermanns were negative.

In spite of the marked improvement in his general condition he complained of extremely severe generalized headaches and some impairment of taste. Improvement was steady and the patient was discharged on the fourteenth hospital day.

The patient's family history, and the manner in which he reacted to the vaccine, suggested strongly that the local reactions and early manifestations of generalized lymph node enlargement might be viewed as an allergic response to the protein contained in the nervous tissue of the vaccine, and that the clinical picture of encephalitis which followed might likewise be an allergic response to the protein of the inocula.

Etiology. Since the case reported by Tonin,[20] the nature of paralytic accidents has been a constant and interesting source of controversy. Koch[11] believed that the paralyses were due to canine rabies. Babes[2] attributed them to a rabies toxin, and Marinesco[15] to the deleterious action of normal nerve substance. França,[8] Fielder,[7] Busson,[5] von Stockum,[21] and others were of the opinion that they resulted from the action of the fixed virus of the inocula. At the Rabies Conference,[14] Calmette supported the theory that paralyses were due to a neurotropic virus contained in the cord of the rabbit and inoculated with the vaccine. Bassoe and Grinker,[3] because of a similarity in the pathologic features of fatal neuropara-

lytic accidents associated with the administration of the Pasteur treatment, and those of non-vaccinal encephalomyelitis found in variola, measles, and other virus diseases, believed they were "dealing with an inflammatory disease caused by an attenuated virus." For similar reasons Marsden and Hurst[16] were of the opinion that paralyses were caused by a "single clinical and pathologic entity," namely, a virus. Remlinger[14] and Stuart and Krikorian[19] subscribed to a "cytotoxic" theory and believed that the reactions resulted from "the introduction of an excessive amount of foreign nerve protein" during the course of treatment, and that the nature of this protein must have been changed by its treatment with carbolic acid so as to render it toxic. Stimson[18] and McKendrick (Rabies Conference)[14] believed that the accidents were the result of anaphylactic reactions to the inocula. Prausnitz[12] adhered to the idea that the paralyses were due to the injection of heterogenous nerve substance in specially predisposed individuals, while Cornwall[6] suggested that they were allergic reactions resulting from the injection of foreign nerve protein.

Schwentker and Rivers[17] have found that brain tissue under proper conditions functions as a "complete antigen and is capable of exciting in rabbits the development of complement-fixing antibodies which are organ, rather than species specific." This discovery, they believe, "opens again, for the brain at least, the question of the etiologic rôle of organ-specific antibodies in certain degenerative diseases . . . and strengthens materially the hypothesis that the encephalomyelitis which follows anti-rabies vaccination is in some manner associated with the development of specific antibodies for brain."

The case just cited presents several features which are worthy of emphasis. A previously healthy person of regular habits suffered reaction to the Pasteur treatment; there was a marked individual and family history of allergic disease; the local reactions were of the severe delayed tuberculin-like type and were associated with a constitutional reaction; and superimposed upon this there developed the clinical picture of encephalitis. These features were sufficient to suggest that the reaction was in the nature of an allergic response to the protein of the inocula and suggested the following investigations.

Investigation. A survey was made of 16 persons known to have had severe accidents as the result of treatment. Each individual was interviewed personally and questioned with regard to the following points: (*a*) the presence of an individual or family history of allergic disease; (*b*) the type of local reaction and its relation to the beginning of the treatment; (*c*) the type of accident, its symptomatology and relation to the beginning of treatment; (*d*) the total number of injections given; and (*e*) the degree of recovery following the accident. In addition, such factors as age, race, occupation,

site of exposure, type of local treatment, and the presence of the so-called "predisposing factors," such as physical or mental strain, nervous or mental disease, living conditions, use of alcohol or drugs, and a history of syphilis were considered.

As a result of this survey it was found that 80% of these patients gave a personal history of asthma, hay fever, urticaria, or marked food idiosyncrasies; that there was a positive family history of allergic disease in 50% of the cases; and that there was either an individual history, family history, or both in 87.5% of the patients interviewed. The local reactions in 80% of the cases were of the severe delayed type described under Group 3, and the remaining 20% were of the delayed type of Group 2. The local reactions began in the majority of the cases about the third or fourth day after treatment had begun, became progressively more severe, and persisted throughout the time that the vaccine was administered. Evidence of neuro-dysfunction began on an average of 15 days after treatment was started. Recovery had occurred, or was occurring, in all but one case (Table 1, Case 4). Of those from whom no allergic history was obtained, one (Table 1, Case 6) drank alcohol excessively throughout the course of treatment, and the other (Table 1, Case 11) was a child of unknown paternity and consequently no reliable family history could be obtained. The results are summarized in Table 1.

TABLE 2.—ALLERGY INCIDENCE IN THOSE RECEIVING RABIES PROPHYLAXIS.

	Personal history of allergic disease, per cent.	Family history of allergic disease, per cent.	Personal and (or) family history of allergic disease, per cent.	No history of allergic disease, per cent.
Sixteen persons suffering treatment accidents	80	50	87.5	12.5
Forty-five persons not suffering treatment accidents	30	20	33.3	66.6

Following the same procedure as was used in taking histories from those who suffered treatment accidents, a control series of 45 persons who had taken the Pasteur treatment without complications were interviewed. The cases were not selected. From this study there developed 4 interesting facts: (1) a positive individual allergic history was obtained in 30%; (2) there was a positive family allergic history in 20%; (3) there was either an individual history, family history, or both in 33% of those interviewed; and (4) there was a rather marked correlation between the severity of the local reactions and the presence of a positive history of allergic diseases.

In comparing these percentages of both groups, it will be seen that in those suffering unfavorable reactions to treatment a history of allergic disease, either personal or familial, or both, was obtained in 87.5% of the cases. In the control series, a similar history was obtained in only 33.3% of those questioned. From this it would

appear that the much higher incidence of allergic disease in the group suffering treatment accidents, in contrast to the group taking the treatment without the development of accidents, was more than a matter of chance, and that allergy played a rôle in the development of treatment accidents (Table 2).

Methods and Results of Desensitization. The occurrence of neuroparalytic accidents is not confined to the use of anti-rabies vaccine, for they are known to occur following the use of typhoid vaccine and various types of serotherapy. Kennedy,[10] Young,[22] Gayle,[9] and Allen[1] have reported such cases and the clinical manifestations are similar to those of the accidents which occasionally attend the administration of the Pasteur treatment.

These, and other reactions to vaccines and serums, are generally thought to be due to foreign protein, and, especially in the case of serum therapy, several well recognized procedures are followed in an effort to prevent their development. First, patients are skin-tested, and if found to be sensitive are desensitized. Second, if during the course of treatment they acquire a sensitivity to the inocula, treatment is stopped and a process of desensitization is begun. Following these simple procedures the inocula may be given with relative impunity and the majority of reactions to treatment avoided. Recently we have given the Pasteur treatment to several persons and have had the opportunity of observing and applying these procedures to those who were or became sensitized to the inocula. The procedure was as follows:

1. Patients to whom the treatment was to be given were skin tested with a 1 to 10 dilution of the No. 1 cord. If a typical allergic wheal developed rapid desensitization was begun, the injections being given every 15 minutes, starting with 0.2 cc. of 1 to 10,000 dilution of No. 1 cord, and continued until the undiluted vaccine was given without reaction.

2. Patients who became sensitive during the course of treatment were given a more gradual method of desensitization, the initial desensitizing dose being determined by that dilution which was tolerated without a local reaction or inciting a reaction at a previous injection site. Daily injections, beginning with 0.5 cc. of the diluted vaccine, were given until the undiluted vaccine was tolerated.

TABLE 1.—SUMMARY OF 16 PATIENTS SUFFERING UNFAVORABLE REACTIONS TO THE PASTEUR TREATMENT.

Case 1.—Mr. W. T., university student, aged 25, was bitten by a dog whose behavior was suggestive of rabies. Laboratory examination of the animal's brain was unsatisfactory. The patient's history was of interest in that several members of his family were "very nervous and highly strung," and that there was an individual and family history of allergic disease. His habits were regular and he denied venereal disease. He had never before taken the Pasteur treatment. Treatment was started on August 6th, 1935. Approximately 40 minutes after the first injection the patient returned complaining of "itching rash" over his face and body. Examination revealed many urticarial wheals varying from 2 to 4 cm. in diameter. The injection site was red and indurated and measured 8 cm. in diameter. Adrenalin,

TABLE 1.—SUMMARY OF 16 PATIENTS SUFFERING UNFAVORABLE REACTIONS TO THE PASTEUR TREATMENT.

Patient.	Sex.	Age.	Race.	Animal.	Number of days between exposure and treatment.	Interval between beginning of treatment and development of			Number of injections given.	Type of paralysis.	Result.	Allergic history.		Remarks.
						Local reaction.		Paralysis.						
						No. days.	Type.	No. days.				Individual.	Family.	
1 H. B.	M.	25	W	Rabid cow	3	4	Delayed severe	19	18	Transverse myelitis	Recovery	Pos.	Pos.	
2 N. L.	F.	22	W	Rabid cat	4	4	Delayed severe	12	14	Transverse myelitis	Recovery	Pos.	Pos.	
3 V. E.	F.	12	W	Rabid dog	7	6	Delayed severe	13	11	Transverse myelitis	Recovering	Pos.	Pos.	Her only allergic manifestation is a violent reaction to horse serum.
4 G. W.	M.	48	W	No exam. dog	1	About 6	Not known to informant	11	9	Ascending paralysis of the Landry type	Death on 15th day	Pos.	Pos.	This was the 2d course of treatment. First was 2 yrs. before.
5 R. F.	F.	24	W	Rabid dog	2	3	Delayed severe	13	16	Loss of sphincter control; weakness of extremities (marked)	Recovering	Pos.	Neg.	
6 R. D.	M.	35	W	Rabid dog	4	2	Delayed severe	12	8	Transverse myelitis	Slight residual weakness	Neg.	Neg.	Used alcohol daily and excessively throughout treatment.
7 E. M.	M.	35	W	Rabid hog	1	About 3	Delayed slight	25	21	Transverse myelitis; unilateral paralysis of Cr. VII	Slight residual weakness	Pos.	Neg.	
8 C. I.	M.	55	W	No. exam. dog	10	5	Delayed severe	19	9	Transverse myelitis	Slight residual weakness	Pos.	Neg.	
9 B. S.	M.	3	W	Rabid cat	4	2	Delayed severe	21	21	Paralysis laryngeal branches of Cr. X	Recovery	Neg.	Pos.	
10 H. H.	M.	22	W	Doubtful cat	6	5	Delayed severe	13	12	Encephalitis	Recovery	Pos.	Pos.	
11 J. G.	M.	17	C	Rabid dog	4	?	Delayed slight	28	21	Transverse myelitis	Recovering	Neg.	Neg.	Father unknown to patient or mother.
12 R. M.	M.	48	W	Rabid dog	1	3	Delayed severe	14	14	Transverse myelitis	Slight residual weakness	Pos.	Neg.	
13 J. B.	M.	26	W	Rabid dog	0	1	Delayed moderate	14	14	Transverse myelitis	Slight residual weakness	Pos.	Neg.	Both parents died during patient's childhood, hence no knowledge of family allergic disease.
14 J. L.	M.	40	W	Rabid dog	5	?	Delayed severe	15	15	Transverse myelitis	Slight residual weakness	Pos.	Neg.?	Drank upon occasions during course of treatment.
15 C. S.	M.	32	W	Rabid dog	1	3	Delayed severe	24	21	Facial	Recovery	Pos.	Pos.	
16 F. B.	M.	25	W	Rabid dog	3	5	Delayed severe	12	15	Transverse myelitis	Slight residual weakness	Pos.	Pos.	
Ave.	M 8:1	29.3	..	80% known rabid	3.5	3.7	Delayed severe	16.5	14.7					

Pos. family allergic hist., 50% Pos. individual allergic hist., 80% Pos. family or individual hist., 87.5%

0.5 cc., was injected with prompt disappearance of the wheals. On the following day desensitization was begun. Injections were given every 15 minutes starting with 0.2 cc. of a 1 to 10,000 dilution of anti-rabic vaccine and ending with 0.5 cc. of the undiluted vaccine. On August 8, 9, and 10 the procedure was repeated, beginning with dilutions of 1 to 1000, 1 to 100, and 1 to 10 respectively. At no time was there a recurrence of the original complaints. He remained desensitized and the subsequent course of treatment was uneventful.

CASE 2.—Miss F. P., university student, aged 21, was bitten by a dog and insisted upon taking treatment. No examination of the animal was made. Treatment was begun in April, 1936. Her history was interesting in that she had been vaccinated against poliomyelitis (Kolmer method) in September, 1935. A skin test with 0.2 cc. of a 1 to 10 dilution of the anti-rabic vaccine resulted in the prompt development of a large erythematous wheal. A process of desensitization was used similar to Case 1. The subsequent course of treatment was wholly without discomfort or reaction and at no time did she regain her sensitivity.

CASE 3.—Mrs. M., housewife, aged 34 was bitten by a dog proved by the State Health Laboratory to have been rabid. She was subject to annual attacks of hay fever. Pasteur treatment was advised. A skin test with 0.2 cc. of 1 to 10 dilution of the vaccine was negative, and the treatment was started. All injections were given in the back. After the third injection there developed a tender erythematous and indurated area at the injection site. Subsequently, local reactions rapidly became more severe, and each injection gave rise to a reaction at the previous injection site. At the time of the sixth injection these areas measured 10 to 12 cm. in diameter, were very firm, had a well demarcated margin, and were exquisitely tender. The treatment was stopped and desensitization begun, using the following plan:

1st day, 0.5 cc. 1:100 dilution of the vaccine.
2d day, 1.0 cc. 1:100 dilution of the vaccine.
3d day, 0.5 cc. 1:10 dilution of the vaccine
4th day, 1.0 cc. 1:10 dilution of the vaccine.
5th day, 0.5 cc. undiluted vaccine.
6th day, 1.0 cc. undiluted vaccine.

Under this plan the local reactions completely disappeared, and the regular treatments were resumed. After 2 injections, however, she regained to a slight degree her sensitivity, and the procedure outlined above was repeated starting with 0.5 cc. of a 1 to 10 dilution. The remaining eleven injections were given without any local reaction or discomfort.

Discussion. Burky and Henton[4] have found that repeated injections of lens extract plus staphylococcus toxin gave rise to a skin sensitivity to lens extract, and that if the lens of a sensitized animal was injured by needling, there developed an intra-ocular inflammation that clinically and histologically resembled endophthalmitis phaco-anaphylactica. This inflammation was believed to be due to the absorption of lens protein by the hypersensitive subject. A method of desensitization was devised (using lens extract and staphylococcus toxin) and successfully applied in causing a remission of the signs and symptoms of experimental and clinical endophthalmitis phaco-anaphylactica. It was noticed by these observers that as their subjects lost their skin sensitivity there was a coincidental

improvement in the ocular signs which, they suggested, might be due to a diminished ocular sensitivity.

Schwentker and Rivers[17] have demonstrated that nervous tissue is capable of exciting the development of organ-specific antibodies and that this tissue became a more effective antigen when it was altered by the addition of pig serum, treatment with vaccine virus, and especially by autolysis. They also showed that, as the result of the injection of nervous tissue into rabbits, the number of paralyses that developed paralleled the antigenic power of the emulsion used. They concluded that "these observations cannot be considered evidence that the paralyses are directly related to the antigenicity of the brain tissue emulsion because, as Hurst has pointed out, . . . they might have been induced by some toxic substance in the autolyzed or diseased brain."

In many respects the experiments of Schwentker and Rivers[17] are analogous to those of Burky and Henton,[4] namely, that an organ-specific substance plus a toxic substance occasioned the development of a sensitivity to the specific substance.

The analogy just made may be carried a step further and applied to an understanding of the reactions that occasionally attend the administration of the anti-rabic vaccine. The nervous tissue that is injected has been altered by infection with a fixed rabies virus and by desiccation. The severe local reactions preceding the onset of the symptoms of central nervous system involvement are a rough index of the degree of acquired sensitivity to the inocula. It is possible to desensitize those who are, or who become sensitive to the inocula and thus cause a remission of the signs of cutaneous hypersensitiveness. It is possible that with the loss of skin sensitivity there may also be a loss, or diminution, of a central nervous system sensitivity which would prevent or arrest the development of a reaction to the treatment.

The exceedingly high incidence of allergic disease among those suffering reactions to treatment would warrant one's questioning each candidate for the treatment concerning a personal or family history of allergy, not alone because accidents are more frequent among these individuals, but because of the severity of the local reactions they are apt to suffer. It seems advisable, therefore, that those who show or develop a marked sensitivity to the inocula should be given the benefits of desensitization.

Summary. The number of neuro-paralytic accidents among those taking the Pasteur anti-rabic treatment has been reported by McKendrick[13a,b] as approximating 0.027%, whereas the North Carolina State Board of Health, in its more limited survey, reports an incidence of 0.083%. In North Carolina the mortality rate of those suffering paralytic accidents was 10% as compared to 16% recorded by Remlinger.[14]

The various types of reactions to the Pasteur treatment may be divided into 6 groups. To these is added the report of a patient who developed encephalitis without paralysis during the Pasteur treatment and which was believed to be due to an allergic response to the inocula.

As the result of an investigation of 16 persons who had suffered reactions to the Pasteur treatment it was found that there was an exceedingly high incidence of allergic disease (87.5%) as compared with a control group (33.3%) in which no neuro-paralytic accidents occurred. In the control group, the allergic individuals suffered much more severe local reactions than the non-allergic individuals.

The various theories concerning the etiology of the paralytic accidents are reviewed, showing the controversial nature of this subject.

This study suggests that a careful investigation of a patient's allergic background and a determination of his sensitivity to the anti-rabic vaccine are valuable measures in determining those individuals who may suffer reactions to the treatment.

A method of desensitization was successfully employed in 3 cases that had, or developed, a marked sensitivity to the anti-rabic vaccine.

A hypothetical analogy is drawn between endophthalmitis phacoanaphylactica, and the unfavorable reactions to the Pasteur treatment. It is suggested that as the result of desensitization, evidenced by the loss of skin sensitivity, there may likewise occur a loss of central nervous system sensitivity. Such a loss of sensitivity would prevent or arrest the development of neuro-paralytic accidents associated with the Pasteur treatment.

REFERENCES.

(1.) **Allen, I. M.**: Lancet, **2**, 1128, 1931. (2.) **Babes, V.**: Internat Beitr. z. inn. Med. Zum 70 Jahr. Geburtstage von Leyden, Berlin, Hirschwald, 1902. (3.) **Bassoe, P.**, and **Grinker, R. R.**: Arch. Neurol. and Psychiat., **23**, 113, 1930. (4.) **Burky, E. L.**, and **Henton, H. C.**: Am. J. Ophth., **19**, 782, 1936. (5.) **Busson, B.**: Wien. klin. Wchnschr., **39**, 1183, 1926. (6.) **Cornwall, J. W.**: Indian J. Med. Res., **6**, 237, 1918–1919. (7.) **Fielder, F. S.**: J. Am. Med. Assn., **66**, 1769, 1916. (8.) **Franca, C.**: Centralbl. f. Bakt., Abt. I, Orig., **55**, 154, 1910. (9.) **Gayle, R. F.**: J. Nerv. and Ment. Dis., **78**, 221, 1933. (10.) **Kennedy, F.**: Am. J. Med. Sci., **177**, 555, 1929. (11.) **Koch, J.**: Centralbl. f. Bakt., Abt. I, **104**, 381, 1927. (12.) **Lubinski, H.**, and **Prausnitz, C.**: Ergebn. d. Hyg. Bakt., Immunitätsf. u. Exp. Ther., Fortsetz. d. Jahr. über die Ergebn. d. Immunitätsf., Berlin, Julius Springer, vol. **8**, 1926. (13.) **McKendrick, A. G.**: (*a*) Health Organization, League of Nations, Geneva, iii, Health, 1930, iii, 2, C. H. 844, p. 33; (*b*) Quart. Bull. Health Organization, League of Nations, Geneva, **1**, 110, 725, 1932. (14.) **Marie, A. C., Remlinger, P.**, and **Valée, H.**: League of Nations Rep. of Internat. Rabies Conf., p. 70, 1927. (15.) **Marinesco, G.**: Compt. rend. Soc. de biol., **64**, 973, 1908. (16.) **Marsden, J. P.**, and **Hurst, E. W.**: Brain, **4**, 181, 1932. (17.) **Schwentker, F. F.**, and **Rivers, T. M.**: J. Exp. Med., **60**, 559, 1934. (18.) **Stimson, A. M.**: J. Med. Res., **23**, 55, 1910. (19.) **Stuart, G.**, and **Krikorian, K. S.**: Ann. Trop. Med. and Parasitol., **22**, 327, 1928. (20.) **Tonin, S.**: Compte rendu statistiqu de l'Institut Antirabic du Caire, 1899–1901, Le Caire, Costogliola, 1902. (21.) **von Stockum, M. J.**: New Principles of Antirabic Treatment, Hague, Martinus Nyhoff, 1936. (22.) **Young, F.**: J. Am. Med. Assn., **98**, 1139, 1932.

STUDIES ON CALCIUM CREOSOTATE.

IV. Observations on Its Use in Pulmonary Tuberculosis.*

By Edwin J. Fellows, Ph.D.,
INSTRUCTOR IN PHARMACOLOGY, TEMPLE UNIVERSITY SCHOOL OF MEDICINE, PHILADELPHIA, PA.

(From the Department of Pharmacology, Temple University School of Medicine.)

Previous experimental work[6a,b] has shown that calcium creosotate compared closely with the creosote from which it was prepared although slight chemical differences[6c] existed between the two drugs. Creosote and its preparations receive widespread application in the treatment of pulmonary disorders despite the fact that a satisfactory explanation of their action has not been advanced. Statements are to be found that under certain conditions[5] expectoration is lessened; while under certain other conditions, the secretion of mucus is believed[12] to be increased during their administration. At the present time, there is a discrepancy of opinion as to the value of creosote and its preparations in the treatment of pulmonary diseases. Recently, Brown[2] has stated that calcium creosotate is of value in chronic inflammatory conditions and that it is especially useful when the sputum is abundant as in lung abscess, bronchiectasis, and suppurative pneumonitis. On the other hand, the Council on Pharmacy and Chemistry of the American Medical Association stated[1] that all creosote preparations were to be omitted from New and Nonofficial Remedies because they believed their use was based purely on empiricism.

The primary purpose of the present investigation was to determine whether or not any modification of 24-hour sputum specimens could be demonstrated during a period of calcium creosotate administration. Patients with pulmonary tuberculosis have been selected because it has been possible to collect reasonably constant amounts of sputum from these individuals, whereas in most bronchial diseases the daily variation in sputum volume is so great that it is impossible to obtain reliable control data. In the present experiments, the volatile phenol content of 24-hour sputum specimens has been investigated both before and during periods of drug administration to determine the presence or absence of the drug in contact with the bronchial mucosa. Estimations of the volatile phenols excreted in urine of patients receiving calcium creosotate have been made as an indication of its absorption. Attempts also have been made to detect phenolic substances in the expired air of experimental animals after calcium creosotate had been administered.

Method. The sputum data in this communication have been obtained from 3 series of hospitalized patients.† Each series as nearly as possible

* The expense of this investigation was defrayed, in large part, by a grant from The Maltbie Chemical Company, Newark, New Jersey.

† All cases referred to in this communication were patients at Eagleville Sanatorium, Eagleville, Pa.

was made up of individuals showing similar degrees of lung change. The procedure has been to compare data secured during satisfactory normal periods of observation with that obtained during drug administration. Approximately one-half the patients of a given series were continued as controls.

Twenty-four-hour sputum specimens were collected in bottles and their volumes estimated therein by comparison against a measured quantity of water. Prior to the phenol determination all specimens were autoclaved at 20 pounds pressure for 40 minutes. Control experiments in which known amounts of creosote were added to 24-hour specimens just before autoclaving demonstrated that volatile phenolic material was not lost in this process. Each autoclaved specimen was treated with 15 to 20 cc. of concentrated phosphoric acid (85%) and steam distilled until 250 to 300 cc. of distillate had been collected. Due to the small amounts of volatile phenols present in the 24-hour specimens it was necessary to extract the distillates, previously acidified by the addition of a few drops of HCl, with ethyl ether. Three extractions of each distillate with 50-cc. portions of ether invariably resulted in a phenol free aqueous layer. The ether was then evaporated, the residue taken up with 60 cc. of distilled water and this treated with 10 cc. of para-diazo-nitraniline. After 3 minutes it was made alkaline by adding 5 cc. of 10% NaOH solution and compared in a colorimeter against a known creosote solution treated in the same manner. Concentrations of 1 to 2 mg. of creosote per 100 cc. when treated with diazotized nitraniline reagent and made alkaline will give a red violet color. Solutions containing 0.2 to 0.5 mg. creosote per 100 cc. will give an orange yellow color. When the diazotized nitraniline reagent itself is made alkaline a green yellow color is produced. Most sputum specimen distillates gave an orange yellow color the intensity of which was seemingly dependent upon the amount and character of the sample distilled. This color has been attributed to volatile phenols but it must be said that no attempt was made to determine the nature of this material. Phenol concentrations of less than 0.2 mg. have been designated in the tables as traces.

Determinations of total volatile phenols eliminated in urine during 24 hours by 4 of the patients receiving four 0.25 gm. calcium creosotate tablets 3 times a day were made in the manner previously described.[6b]

Results and Discussion. Results of the observations made on the first series of patients examined have been placed in Table 1. Each sputum volume recorded in this table is an average of 30 determinations. The sputum volatile phenol values in this and all succeeding tables represent the highest and lowest obtained either before or during a period of drug administration. The total period of observation for this series extended over 3 months; the actual time of drug administration was 1 month. In this and all other series the patients were given an initial dose of one 0.26-gm. calcium creosotate tablet 3 times a day. The amount of the drug given was gradually increased if the patients were not nauseated by this initial dose. The drug was always administered after meals with a copious amount of water. In this series, it was impossible to give more than 2 tablets 3 times a day. After the drug had been given for a period of 1 month it became necessary to discontinue this series because practically every individual of this group was nauseated. It is to be noted that the sputum phenols of the patients receiving the drug did not increase during this period. In computing the average

normal sputum volumes it was observed that most of the specimens remained remarkably constant from day to day.

The grand average of the sputum volumes of those patients receiving the drug shows a loss of 1.7 cc., whereas that of the control patients shows a loss of 2.09 cc. during the same period. In other words, the sputum volumes of both groups remained remarkably constant. The character of the 24-hour sputum specimens was described each day but insufficient change was noted in any case to warrant tabulation of the data.

TABLE 1.—VOLATILE PHENOLS AND VOLUME OF SPUTUM. FIRST SERIES.

Receiving drug.	24-hour sputum volumes—average of 30 observations.		24-hour sputum volatile phenols.	
	Before drug administration, cc.	During drug administration, cc.	Before drug administration, mg.	During drug administration, mg.
V. G.	3.0	4.2	traces	traces
J. C.	4.1	4.5	traces	traces
L. M.	5.1	6.9	tr.–0.2	traces
F. H.	12.8	14.3	tr.–0.2	tr.–0.2
S. B.	6.0	5.2	traces	traces
T. K.	12.8	10.0	tr.–0.2	tr.–0.2
A. G.	4.6	2.8	traces	traces
X. K.	6.0	4.0	traces	traces
C. M.	5.0	5.3	traces	traces
Z. M.	14.5	10.4	tr.–0.2	tr.–0.2
L. ST.	2.4	2.1	traces	traces
W. K.	75.0	69.0	0.5–0.6	0.5–0.7
V. M.	121.0	110.0	0.6–0.8	0.6–0.8
J. L.	28.2	28.0	tr.–0.4	0.2–0.4
Grand average	21.4	19.7		
Controls.				
A. B.	3.3	4.7	traces	traces
A. L.	48.5	25.5	0.3–0.5	0.4–0.5
L. R.	25.3	26.0	tr.–0.4	tr.–0.4
T. B.	52.4	46.2	0.2–0.3	tr.–0.3
H. F.	37.5	36.0	0.2–0.3	tr.–0.3
J. M.	3.7	3.2	traces	traces
L. Sp.	31.0	39.7	tr.–0.3	tr.–0.2
P. S.	18.6	20.6	tr.–0.3	tr.–0.3
H. G.	3.8	6.0	traces	traces
E. R.	10.7	6.0	traces	traces
Grand average	23.48	21.39		

The control observations of the second series extended over 3 months. One-half of this group then received the drug for 5 consecutive months. Each sputum volume recorded in Table 2 for patients of this series is the average of 50 determinations. No significant variations are to be noted in the group of patients receiving the drug as compared with the control group. This applies to individual patients as well as to the grand averages. It is to be noted also that in this group the sputum volatile phenols did not increase during the period of drug administration. These data should be accepted as conclusive because the majority of the patients

receiving the drug were given four 0.26-gm. tablets 3 times a day throughout the 5 consecutive months. After a control period of 7 days the patients of this series receiving calcium creosotate were given 0.3-gm. terpin hydrate 3 times a day for 7 days. It is to be noted in Table 3 that no significant change in volume was observed during this period.

TABLE 2.—VOLATILE PHENOLS AND VOLUME OF SPUTUM. SECOND SERIES.

Receiving drug.	24-hour sputum volumes—average of 50 observations.		24-hour sputum volatile phenols.	
	Before drug administration, cc.	During drug administration, cc.	Before drug administration, mg.	During drug administration, mg.
F. H.	14.0	13.0	tr.–0.35	0.2–0.3
M. T.	20.5	18.2	0.25–0.4	tr.–0.4
L. S.	2.3	5.8	traces	traces
M. M.	4.3	5.9	traces	traces
W. M.	18.3	21.4	tr.–0.2	tr.–0.2
A. D.	23.7	24.1	tr.–0.25	tr.–0.2
R. T.	32.4	26.3	0.25–0.35	tr.–0.03
C. A.	41.7	40.4	0.4–0.5	0.3–0.5
L. M.	16.2	19.1	0.2–0.3	tr.–0.3
J. C.	5.4	6.0	traces	traces
N. H.	37.4	31.1	0.25–0.35	0.2–0.3
M. S.	16.1	15.7	0.2–0.3	tr.–0.3
D. B.	8.3	13.4	tr.–0.2	0.2–0.25
L. Si.	24.1	28.0	0.2–0.3	0.2–0.4
L. Sp.	81.8	72.6	tr.–0.3	tr.–0.2
Grand average	22.8	22.7		
Controls.				
V. G.	4.6	5.1	traces	traces
T. B.	27.5	29.9	0.2–0.25	0.2–0.25
J. L.	28.0	29.9	0.25–0.35	0.2–0.3
L. M.	10.7	10.4	0.2–0.25	tr.–0.2
E. M.	50.1	41.0	0.3–0.45	0.2–0.4
F. M.	8.0	11.8	traces	traces
R. R.	7.4	9.0	0.2–0.3	tr.–0.3
I. M.	30.4	24.0	tr.–0.3	0.2–0.3
M. L.	3.0	4.0	traces	traces
A. G.	2.4	2.6	traces	traces
J. Cl.	4.7	3.6	traces	traces
H. F.	50.4	44.0	0.2–0.3	0.2–0.4
G. M.	5.0	6.1	traces	traces
A. B.	5.3	6.6	traces	traces
P. S.	24.1	25.8	0.2–0.3	tr.–0.3
J. W.	8.2	13.7	0.2–0.3	tr.–0.3
D. S.	8.3	7.5	traces	traces
Grand average	16.36	16.17		

Data also were obtained from 18 patients who were soon to be discharged. It was believed that the individuals of this series would show less tendency to become nauseated than the members of the previous groups. The opposite proved to be true, so that at the end of 4 weeks this series had to be discontinued though the amount of drug administered was only one or two 0.26-gm. tablets 3 times a day. As in the previous groups investigated, the variations

in sputum volumes shown by patients receiving the drug were no greater than the variations shown by the control patients. The sputum phenols also were not increased during the period of drug administration. It is possible that statements in the literature concerning the sputum volume changes after creosote may be the result of isolated observations on individuals who might have shown the same changes without the drug. In the present experiments, the sputum volumes of certain of the patients increased considerably while in others it decreased during medication. However, it must be emphasized that the same degree of change was noted in the control patients.

TABLE 3.—VOLUME OF SPUTUM. THIRD SERIES.

	24-hour sputum volumes—average of 7 observations.	
Patients.	Before terpin hydrate administration, cc.	During terpin hydrate administration, cc.
F. H.	15.4	23.0
M. T.	11.1	9.1
L. S.	2.1	2.4
W. M.	11.5	9.0
A. D.	14.6	15.3
R. T.	21.5	17.0
C. A.	23.6	23.1
M. M.	4.6	2.8
L. M.	13.8	7.6
N. H.	36.1	37.0
M. S.	11.1	10.2
Average	15.0	14.2

Groner[8] states that the appetites of tuberculous patients are increased by guaiacol and creosote. He believes this is to be explained by an intestinal antiseptic and gastrosecretory stimulant action. In the present investigation no evidence of lessened cough, greater ease of expectoration, or increased appetite was obtained for those individuals receiving calcium creosotate.

TABLE 4.—VOLATILE PHENOLS IN URINE.

	Twenty-four hour specimen—average of 8 determinations.	
Patients.	Before calcium creosotate administration, mg.	During calcium creosotate administration, mg.
J. C.	35	985
A. D.	20	770
N. H.	25	850
R. T.	18	980

A number of individuals have reported that creosote is valueless in tuberculosis but is of value in certain other conditions. Dube, for example,[4] believes creosote of no value in tuberculosis, except

where there are associated coccal infections. In pneumonia, he states the temperature is lowered, the patient breathes more easily, expectoration is less viscous and more easily expelled. McKinley[10] used creosote enemata in lobar pneumonia and states that temperatures were lowered and the crisis hastened. He believes the action of creosote in purely pneumococcic conditions to be almost specific. Merrilles[11] states that creosote is of no value in other than coccal infections. He also believes that in pneumonia and meningitis creosote is more or less specific.

Bufalini[3] reported that phenols were not excreted in the expired air of rabbits after subcutaneous or oral administration of creosote or guaiacol. In his experiments, the expired air was collected in $NaHCO_3$ solution and tested for phenols by adding Lugol's solution and estimating the excess iodine.

Failure in the present experiments to find evidence of excretion of phenols in the sputum specimens of those individuals receiving calcium creosotate suggested the necessity of investigating expired air of animals given the drug. Each of 6 rabbits was given by stomach tube 0.5 gm. of water soluble calcium creosotate phenols, muzzled, and the expired air collected in a 5% NaOH solution for periods of 8 hours in some cases, to 18 hours in others. At the end of these intervals the absorption liquid was neutralized with HCl, para-nitraniline reagent added and made alkaline with NaOH solution. A very slight darkening sometimes took place when the absorption fluid was treated with para-nitraniline reagent, but absorption fluid from control rabbits also exhibited the same phenomenon. Therefore in these experiments no evidence was obtained for the presence of phenolic material in the expired air of animals given calcium creosotate.

Gordonoff[7] has reported experimental demonstrations of an "expectorant" effect after guaiacol. He also has reported finding concentrations of 1 to 5000 to 1 to 10,000 of the drug in extirpated lungs of rabbits previously given from 1 to 1.5 gm. of guaiacol. From his results he concluded that guaiacol exerted an expectorant effect by virtue of a secretory and motor action. Gordonoff failed to report the phenol concentrations in other organs of the body. On the other hand, Hofbauer[9] has reported that when guaiacol is injected hypodermically, the major part is contained in the blood, liver and spleen, much less in the lungs and kidneys.

In our experiments, failure to find evidence of excretion of calcium creosotate phenols in 24-hour sputum specimens during drug administration coupled with the failure to detect phenols in the expired air of animals, leads one to doubt the presence of appreciable amounts of calcium creosotate along the bronchial mucosa. Walters[13] has stated that creosote acts mainly (or entirely) on associated infections in tuberculosis. From the results of the present experiments this is difficult to visualize.

The data obtained for the volatile phenol content of 24-hour urine specimens from 4 of the patients receiving four 0.26-gm. tablets of calcium creosotate 3 times a day have been recorded in Table 4. The figures represent the average values obtained during 8 different days. These values appear to be rather high if one considers only certain phases of previous chemical investigations[6c] on calcium creosotate. In these experiments,[6c] it was shown that approximately one-fourth of calcium creosotate powder was made up of water soluble phenols. On this basis, these patients received in the calcium creosotate tablets approximately 750 mg. of water soluble phenols daily. As shown in Table 4, these patients excreted phenols in excess of this amount. Numerous investigators have shown that only a fractional part of orally administered phenols are excreted in urine. In the work cited above,[6c] in addition to demonstrating that one-fourth of the calcium creosotate powder was made up of water soluble phenolic material, it also was shown that 56% of the drug was made up of organic material. No attempt was made to identify the 30% of water insoluble organic matter. The present high urine phenol values after calcium creosotate tablets suggest the possibility that the water insoluble organic material may be phenolic in nature, soluble in the gastro-intestinal secretions and hence absorbed. Another explanation of the high values may be that since the drug was administered 3 times a day and the 24-hour urine specimens collected only at irregular intervals there may have been overlapping of doses.

The nature of calcium creosotate phenols excreted in human urine was not investigated in the present experiments. In other experiments using rabbits[6d] it was shown that practically all orally administered calcium creosotate was excreted in rabbit urine in conjugated form.

Summary. 1. Administration of calcium creosotate to patients with pulmonary tuberculosis failed to modify the volume or character of their 24-hour sputum specimens.

2. A number of individuals in the groups investigated tolerated large doses (3.1 gm. daily) of calcium creosotate for periods of several months. On the other hand, some were severely nauseated in short intervals by 0.75 gm. daily.

3. Evidence is presented to show that none of orally administered calcium creosotate was excreted in sputum specimens of the patients investigated.

4. No evidence was obtained to indicate that the patients receiving calcium creosotate expectorated more easily, that their cough was lessened or appetites increased.

5. The detection of large amounts of volatile phenols in urine after administration of calcium creosote is evidence of its absorption in man.

6. Volatile phenols could not be detected in expired air of rabbits after calcium creosotate had been administered.

We wish to express our thanks to Dr. Louis Cohen whose efforts have made possible the coöperation between Eagleville Sanatorium and this department. Grateful acknowledgment is also made to Dr. A. E. Livingston for the many helpful suggestions and encouragement given during the course of these experiments.

REFERENCES.

(1.) Am. Med. Assn. Coun. on Pharm. and Chem.: J. Am. Med. Assn., **110**, 209, 1938. (2.) **Brown, C. L.**: Ibid., **109**, 268, 1937. (3.) **Bufalini, G.**: Lo Sperimentale, **58**, 568, 1904. (4.) **Dube, E.**: Union med. du Canada, **62**, 104, 1933. (5.) **Edmunds, C. W.**, and **Gunn, J. A.**: Cushny's Pharmacology and Therapeutics, Philadelphia, Lea & Febiger, p. 773, 1936. (6.) **Fellows, E. J.**: (*a*) J. Pharm. and Exp. Ther., **60**, 178, 1937; (*b*) Ibid., p. 183; (*c*) J. Am. Pharm. Assn., **26**, 609, 1937; (*d*) Studies on Calcium Creosotate, V. A. Attempts to Produce Bacteriostatic Urine in Man and Animals by Oral Administration of Calcium Creosotate. B. The Nature of the Phenols Excreted in Rabbit Urine After Oral Administrations of Creosote and Calcium Creosotate. (To be published.) (7.) **Gordonoff, T.**, and **Jannett, F.**: Ztschr. f. d. ges. exper. Med., **83**, 567, 1932. (8.) **Groner, P.**: Wien. med. Wchnschr., **79**, 886, 1929. (9.) **Hofbauer, L.**: Therap. Monatsh., **29**, 237, 1915. (10.) **McKinley, R.**: J. Roy. Army Med. Corps, **61**, 84, 1933. (11.) **Merrilles, J. F.**: Med. J. Australia, **2**, 157, 1916. (12.) **Sollmann, T.**: A Manual of Pharmacology, 5th ed., Philadelphia, W. B. Saunders Company, p. 617, 1936. (13.) **Walters, F. R.**: Lancet, **1**, 1272, 1927.

THE EFFECT ON THE DEVELOPING RED BLOOD CELLS IN THE FETUS, OF ADMINISTERING HUMAN AND HOG GASTRIC JUICE TO THE ADULT RAT DURING PREGNANCY.

By Joseph Stasney, M.D.,

FELLOW IN PATHOLOGY, THE MAYO FOUNDATION FOR MEDICAL EDUCATION AND RESEARCH, GRADUATE SCHOOL, UNIVERSITY OF MINNESOTA,

George M. Higgins, B.S., M.A., Ph.D.,

ASSOCIATE IN DIVISION OF EXPERIMENTAL MEDICINE, INSTITUTE OF EXPERIMENTAL MEDICINE; ASSOCIATE PROFESSOR OF EXPERIMENTAL BIOLOGY, THE MAYO FOUNDATION FOR MEDICAL EDUCATION AND RESEARCH, GRADUATE SCHOOL, UNIVERSITY OF MINNESOTA,

AND

Frank C. Mann, B.A., M.D., M.A., D.Sc., LL.D.,

DIRECTOR OF THE DIVISION OF EXPERIMENTAL MEDICINE, INSTITUTE OF EXPERIMENTAL MEDICINE; PROFESSOR OF EXPERIMENTAL SURGERY, THE MAYO FOUNDATION FOR MEDICAL EDUCATION AND RESEARCH, GRADUATE SCHOOL, UNIVERSITY OF MINNESOTA.

(From the Division of Experimental Medicine, The Mayo Foundation, Rochester, Minn.)

The hematopoietic factors which control the progressive reduction in the size and volume of the erythrocytes during the development of the fetus and maintain their normal values during adult life are not entirely understood. It is known that antianemic substances occur in adult liver and in gastric juice as well. Castle and his associates[4] expressed the opinion that there is an extrinsic factor in food, which coupled with an intrinsic factor in gastric juice forms the antianemic principle. Morris and his co-workers[16] and Green-

spon[9] attributed the hematopoietic potencies to gastric juice alone. Other workers[5,24,29] have demonstrated antianemic substances in desiccated whole stomach. Meulengracht[14] claimed that desiccated gastric mucosa removed from regions of the pylorus and from areas associated with Brunner's glands contains a potent stimulus for red blood cell stimulation. After total gastrectomy in the hog, the amount of antianemic principle stored in the liver was considerably less than in the normal animal.[1,8] Macrocytic anemia developed in a hog 3 years after total gastrectomy,[1] but Mann and Graham[13] found no changes in the blood picture of a dog 7 years after removal of the stomach. Thus the stomach is not the only source for antianemic principle in all species. Richter, Ivy and Meyer[19] found that desiccated whole stomach of the dog does not contain as much of the antianemic principle as does desiccated stomach of the hog; the principle is nevertheless present in dog's liver. Reimann and his associates[18] failed to demonstrate any hematopoietic factor in gastric juice obtained from a horse, while Richter, Meyer and Ivy[20] showed that it is present in the liver of the horse. Wintrobe, Kinsey, Blount and Trager[32] could not demonstrate an antianemic factor in fetal liver; but Goldhamer, Isaacs and Sturgis[6] stated that it is present in human fetal liver 2 months before birth. Goldhamer and others concluded that, since not all livers contain the active principle, its presence in the liver indicates that it is a storage product.

Bio-assay of the antianemic principle has not given consistent results. Pigeons,[26] guinea-pigs[11] and rats[25] have been employed but results have been contradictory. Singer[23] claimed that rats kept on a standard diet show a constant elevation of the percentage of reticulocytes in response to normal gastric juice administered parenterally. Gastric juice of patients suffering from pernicious anemia, or gastric juice heated to temperatures of 100° C. failed to elicit comparable reticulocyte responses. Gastric juice which had been ultrafiltrated and which was therefore free from proteins, as the negative biuret reaction indicated, did not produce in rats any significant rise in percentage of reticulocytes.[12] Thus the active principle seems in some way bound to protein. Also, it has been shown that the reticulocyte response[7,15,21,22] to antianemic principles is not specific, for increased percentages of reticulocytes have followed the administration of the various hormones and other nonspecific proteins.

The reticulocyte response is accordingly not an entirely reliable criterion for the determination of antianemic potency. It has seemed to us that the physiologic macrocytosis which occurs normally in most fetal mammals may serve as a test object for these principles governing hematopoiesis. The large red cells in the fetus rapidly reduce in size and volume after birth, owing perhaps to the fact that greater amounts of the antianemic principles are then available for red cell stimulation.

During development from birth to maturity the average diameter of the red blood cell in the white rat decreases from approximately 10 microns to about 6. Ordinarily, enlarged erythrocytes do not occur in the healthy adult, but in certain pathologic conditions involving liver damage macrocytoses have been described.[10] Wintrobe and Shumacker[31] observed the similarity between the fetal red blood cells and those of patients with pernicious anemia who were subjected to the influence of liver therapy. This similarity led Wintrobe and Shumacker to suggest that the essential antianemic principles which are required for the maturation of the red cells may not be available in adequate amounts, either in fetal blood or in the blood of patients with pernicious anemia.

If maturation of the red cell is a function of the antianemic principle in gastric juice, it seems reasonable to conclude that augmenting the maternal stores during pregnancy might induce changes in the size and volume of the fetal red cells. Stimulation of erythropoiesis in the fetus is thought to be a function of the anitanemic principle of the mother. Certainly there is no conclusive evidence that the fetus elaborates its own principle. Extracts of fetal liver[2] and of placenta[32] have proved ineffective in producing reticulocyte responses. Wigodsky, Richter and Ivy[28] have recently reported excellent results with an extract prepared from fetal calves' liver. They secured a reticulocytosis of 49% in patients who were in severe relapse with pernicious anemia. There is no evidence to indicate that the developing fetal gastro-intestinal tract elaborates any substances which have hematopoietic potencies.

This report covers the results of our study, in which we have injected concentrated gastric juice of man and of hog into pregnant rats during a part of the period of gestation and have then studied the red blood cells of the young rats at birth.

Materials and Methods. Samples of gastric juice were obtained, following test meals or the administration of histamine, from healthy persons from the Section on Gastro-enterology at The Mayo Clinic. These samples were filtered, pooled, concentrated to one-tenth their original volume and stored in the refrigerator. Before use these samples were neutralized with sodium hydroxide.

Samples of gastric juice free of food factors were obtained from a fundic pouch prepared in the stomach of a young hog, weighing 40 pounds. The pouch was drained by a Pezzar catheter, connected to a rubber balloon. Gastric juice was removed from the balloon 3 times daily, pooled, concentrated and neutralized as were the human samples. All samples of gastric juice used gave a positive biuret reaction.

Samples of gastric juice, both human and hog, which were used for control injections, were heated to 100° C. for 15 minutes.

One cubic centimeter of the concentrated gastric juice, the normal or the heated, was injected daily into the peritoneal space of rats during a part or all of the gestation period. Seventeen pregnant rats received 1 cc. daily of concentrated human gastric juice. Of these, 5 received injections on the 4 days immediately preceding parturition, 5 received injections on the 6 days immediately preceding parturition, and 4 received injections on the

12 days immediately preceding parturition. Three pregnant rats received 1 cc. of the heated gastric juice on each of the 8 days immediately preceding parturition.

Fifteen pregnant rats received 1 cc. daily of concentrated hog gastric juice obtained from the fundic pouch. Of these, 4 rats received injections on the 4 consecutive days preceding parturition, 5 received injections on each of the 7 consecutive days preceding parturition and 2 received injections on each of the 10 days preceding parturition. Four pregnant rats, serving as controls, received injections on the 4 consecutive days preceding delivery, of 1 cc. of the concentrated hog gastric juice which had been heated to 100° C. for 15 minutes.

At birth, blood was taken by cardiac puncture from each newborn rat. The total number of red cells per c.mm. was determined, using standard pipets. Using heparin as an anticoagulant, the percentage of red cells of whole blood was computed. Hematocrit tubes (Van Allen's type) were centrifuged for 15 minutes at 2400 revolutions per minute. Smears were stained with Wright's stain and the red cells were measured directly in microns from photographs made of these smears at 1000 diameters on photosensitized paper. One hundred cells from the blood of each newborn rat were measured. Cell volumes were computed according to the method of Wintrobe.[30]

Results. The data assembled from the study of the blood of newborn rats the mothers of which had received human gastric juice show that human gastric juice contains some principle which when injected into pregnant rats has a definite effect upon the size and volume of the red blood cells of the developing fetus (Table 1). There were slight increases from normal levels in the number of red cells per c.mm. of fluid, decreases in the percentage of packed red cells and decreases in the largest diameters as well as in the volumes of the red cells. In general, the most marked changes were observed in those newborn rats born of mothers which had received an injection each day for the last 12 days of pregnancy. The curves (Price-Jones curves) depicting the percentage distribution of the sizes of the red blood cells in normal rats and in those born of mothers which had received 12 injections of gastric juice are shown (Fig. 1).

The red cells in mature rats of our strain measured 6.1 microns in average diameter, which is just 4 microns less than the average diameter of the red cells of their normal young at birth. The average diameter of cells of rats born of mothers which had received 12 injections was 8.5 microns, or 1.5 micron less than the normal diameter at birth. The decrease in the average diameter of red cells of newborn rats produced by 12 injections of gastric juice into the mother is therefore about 39% of the total decrease in size of red cells which occurs during the entire postnatal development.

When gastric juice was heated to 100° C. for 15 minutes and then injected into pregnant animals, there were no significant effects on the size or volume of the red cells in the offspring (Table 1).

The data on the blood of newborn rats born to mothers which had received the gastric juice from the fundic pouch of the hog (Table 2)

resemble those observed following the administration of human gastric juice, and show that there is present in the hog gastric juice, as in that of man, some substance which hastens maturation of the red cells (Table 1). The effect was proportional to the amount of concentrated juice given the mother. The decrease in the average red cell size in young born to mothers receiving 10 daily injections was about 31% of the total decrease in size which normally occurs during the interval from birth to maturity.

TABLE 1.—EFFECT OF INTRAPERITONEAL INJECTION OF 1 CC. OF CONCENTRATED HUMAN GASTRIC JUICE INTO PREGNANT RATS ON THE SIZE AND VOLUME OF THE ERYTHROCYTES OF THEIR OFFSPRING AT BIRTH OR SHORTLY AFTERWARD.

Daily injections into mother rat.	Newborn animals.	Volume of packed red cells, cc. per 100 cc. of blood.	Erythrocytes. Number per c.mm. millions.	Erythrocytes. Largest diameter, microns.	Erythrocytes. Mean volume, cu. microns.
0	20	43.7 ± 0.98 (39.0 – 53.0)	2.89 ± 0.07 (2.00 – 3.42)	10.09 ± 0.12 (9.67 – 10.79)	151.2
4	25	41.0 ± 0.39 (39.0 – 44.0)	2.81 ± 0.09 (2.25 – 3.19)	9.12 ± 0.09 (8.45 – 9.47)	145.9
6	22	37.3 ± 0.54 (32.0 – 47.0)	3.21 ± 0.05 (2.87 – 3.79)	8.84 ± 0.05 (8.25 – 9.39)	116.2
12	24	39.5 ± 0.77 (37.0 – 45.0)	3.31 ± 0.07 (3.10 – 3.50)	8.54 ± 0.07 (7.93 – 8.98)	119.3
8*	20	41.3 ± 0.53 (34.0 – 46.0)	2.69 ± 0.07 (2.30 – 3.20)	10.01 ± 0.05 (9.82 – 10.19)	153.5

* Inactivated gastric juice was injected.

TABLE 2.—EFFECT OF INTRAPERITONEAL INJECTION OF 1 CC. OF CONCENTRATED HOG GASTRIC JUICE INTO PREGNANT RATS ON THE SIZE AND VOLUME OF THE ERYTHROCYTES OF THEIR OFFSPRING AT BIRTH OR SHORTLY AFTERWARD.

Daily injections into mother rat.	Newborn animals.	Volume of packed red cells, cc. per 100 cc. of blood.	Erythrocytes. Number per c.mm., millions.	Erythrocytes. Largest diameter, microns.	Erythrocytes. Mean volume, cu. microns.
0	20	43.7 ± 0.98 (39.0 – 53.0)	2.89 ± 0.07 (2.00 – 3.42)	10.09 ± 0.12 (9.67 – 10.79)	151.2
4	24	41.4 ± 0.34 (38.0 – 45.0)	2.89 ± 0.06 (2.40 – 3.58)	9.16 ± 0.05 (8.57 – 9.85)	143.7
7	28	40.6 ± 0.66 (32.0 – 46.0)	3.07 ± 0.07 (2.34 – 3.80)	8.99 ± 0.06 (8.15 – 9.59)	133.7
10	7	38.7 ± 0.68 (36.0 – 43.0)	3.21 ± 0.04 (3.00 – 3.50)	8.84 ± 0.11 (8.52 – 9.47)	120.6
4*	16	41.8 ± 0.67 (36.0 – 49.0)	2.82 ± 0.05 (2.08 – 3.40)	9.73 ± 0.05 (9.32 – 10.21)	150.2

* Inactivated gastric juice was injected.

Comment. Gastric juice obtained from man or from the fundic pouch of a hog has been shown to contain some substance which accelerates the maturation of the red blood cells in the developing white rat. This acceleration was demonstrated in the blood of newborn rats by changes in the size and volume of their red cells. This substance in gastric juice is thermolabile, for when the samples of gastric juice were heated before its administration to pregnant rats, no changes in size or volume of the red cells in the young at the time of birth were observed. The effect induced in the red cells of the young rats was proportional to the number of injections of

gastric juice given the pregnant rat, for decreases from normal levels in both size and volume of the red cells were larger in those rats born to mothers which had received the daily injections of gastric juice for longer periods of time.

The physiologic macrocytosis of the newborn rat resembles the pathologic macrocytosis of certain anemias in that both conditions respond to treatment with the antianemic principle. It may be that the principle in gastric juice which reduces the normal physiologic macrocytosis of the newborn rat is identical with or a part of the antianemic principle in liver which is effective in producing remissions of certain macrocytic anemias. Castle and Ham[4] have concluded that the antianemic principle which is stored in the liver is formed by the interaction of a factor in the diet with a factor in the gastric juice. Thus far our data on the effect of liver extract on the normal macrocytosis of young rats are incomplete, but indicate that some reduction in the size of the red cell in the newborn follows the administration of liver extract to the adult rat during gestation. Wigodsky and Ivy,[27] on the other hand, concluded that the erythrocyte picture of newborn rats, born to mothers which were treated with liver extracts of known antipernicious anemia potency, was not significantly different from that of untreated controls. Wintrobe, Kinsey, Blount and Trager[32] did not observe any effect on the red blood cells of fetal rabbits from injecting liver extract into the mothers intramuscularly twice weekly. These authors likewise injected liver extract directly into the placenta at laparotomy. No significant changes were induced in the red cells of the fetus 13 days later at hysterectomy.

The extent of the physiologic macrocytosis which occurs in the blood of newborn animals varies greatly even within a given litter. This suggests that the amount of antianemic principle which is available to the fetus during development may vary from day to day. In all probability the source for the antianemic principle is the mother. There are no data to indicate that the fetus elaborates its own principle, for the extrinsic factor is probably absent and our knowledge concerning the elaboration of the intrinsic factor by the fetal stomach is incomplete. Briese[3] concluded from her study of macrocytosis in newborn rats, the offspring of mothers treated with carbon tetrachloride, that the pathologic condition induced by the drug in the maternal liver seriously restricts the amounts of antianemic principle available to the fetus. This further indicates that the fetus depends on the stores in the maternal liver for its antianemic principle. As these stores are reduced in amount, owing to the cirrhotic condition of the liver, the degree of fetal macrocytosis seems to increase. Recently Parsons and co-workers[17] studied the effect of diets deficient in iron on the size of the red cells in rats and they observed a microcytic hypochromic anemia in the offspring of these rats.

It is of interest that human and hog gastric juice proved equally effective in producing changes in the sizes and volumes of the red cells of newborn rats. Obviously human gastric juice, taken after test meals or histamine injections, included both intrinsic and extrinsic factors in its composition. But hog gastric juice removed by catheter from a separate pouch was necessarily devoid of any food factor and thus contained essentially the intrinsic factor only. However, it is possible that secretions from such a pouch may contain substances like the extrinsic factor, which are derived from gastric mucosa. An investigation of this possibility was beyond the scope of our present study. In both samples of gastric juice the active substances were thermolabile, so that we are inclined to believe that

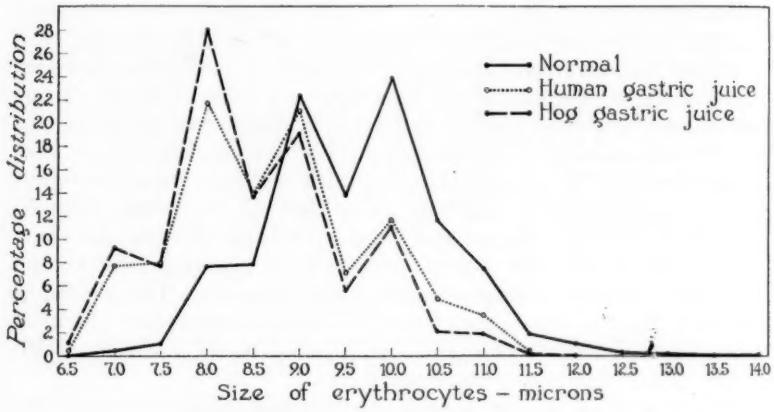

FIG. 1.—Percentage distribution, according to size, of the erythrocytes of rats born of untreated mothers, of mothers who had received human gastric juice for the last 12 days of pregnancy and of mothers who had received hog gastric juice for the last 10 days of pregnancy.

this principle, found in gastric juice, which reduces the size of red cells is either identical with the intrinsic principle of Castle or related to it.

No adequate bio-assay of the antianemic principle has yet been described. The reticulocyte response has been employed but there are serious objections to its use. In the first place the response is nonspecific, for rises in reticulocyte percentages may be obtained in response to all sorts of substances.[12,21] And in the second place normal animals often show spontaneous unpredictable elevations in percentage of reticulocytes, so that any interpretation of results following the administration of antianemic substance is difficult.[7] It is suggested that changes in the size of the red cell of the fetal or newborn rat may be a means of assaying antianemic potency of substances. The reaction is definite and the extent of the decrease

in size of the cells seems correlated with the amount of the principle administered. More experimental investigations are necessary to test the reliability of this method for bio-assay.

Summary and Conclusions. Concentrated samples of human and hog gastric juice have been administered daily to pregnant rats during a part of the gestation period. A study of the blood (obtained by cardiopuncture) of the young rats born to these adults has been made as soon after parturition as possible. Observations were made on the total number of red cells per c.mm., the volume of packed cells per 100 cc. of blood, the greatest diameter of the red cells and the volumes of the red cells. These data have been contrasted with comparable data obtained from young rats born to mothers which received inactivated samples of gastric juice and also with data obtained from young born to untreated normal adults. The following conclusions have been made:

1. Human gastric juice, obtained after test meal or histamine injection, and hog gastric juice, obtained from a fundic pouch, contain a substance which, when administered to the pregnant adult rat, accelerates the reduction in the size and volume of the developing red blood cells of the newborn rat. This substances causes: an increase in the number of red cells per c.mm. of fluid; a decrease in the percentage of packed red cells; a decrease in the greatest diameter of the red cells, and a decrease in the volume of the red cells.

2. These changes induced in the red cells of the newborn rat are, as a rule, proportional to the amount of gastric juice administered to the adult rat during gestation.

3. Heating samples of gastric juice renders the substance ineffective.

4. The physiologic macrocytosis which occurs normally in mammalian fetuses may be due to an inadequate amount of the antianemic principle, which is provided by the mother.

5. This effect upon the size and volume of red blood cells may prove to be a means of assaying the potency of antianemic principle.

REFERENCES.

(1.) **Bence, J.**: Ztschr. f. klin. Med., **130**, 275, 1936. (2.) **Berglund, H., Watkins, C. H.,** and **Johnson, R.**: Proc. Soc. Exp. Biol. and Med., **25**, 834, 1928. (3.) **Briese, E.**: Am. J. Med. Sci., **195**, 787, 1938. (4.) **Castle, W. B.,** and **Ham, T. H.**: J. Am. Med. Assn., **107**, 1456, 1936. (5.) **Conner, H. M.**: Ibid., **94**, 388, 1930. (6.) **Goldhamer, S. M., Isaacs, R.,** and **Sturgis, C. C.**: Am. J. Med. Sci., **188**, 193, 1934. (7.) **Goodman, L., Geiger, A. J.,** and **Claiborn, L. N.**: Proc. Soc. Exp. Biol. and Med., **32**, 810, 1935. (8.) **Goodman, L. S., Geiger, A. J.,** and **Klumpp, T. G.**: J. Clin. Invest., **15**, 435, 1936. (9.) **Greenspon, E. A.**: J. Am. Med. Assn., **106**, 266, 1936. (10.) **Higgins, G. M.,** and **Stasney, J.**: Folia hæmatol., **54**, 129, 1936. (11.) **Jacobson, B. M.**: J. Clin. Invest., **14**, 665, 1935. (12.) **Klaperzak, J.**: Folia hæmatol., **56**, 233, 1936. (13.) **Mann, F. C.,** and **Graham, A. S.**: Ann. Surg., **95**, 455, 1932. (14.) **Meulengracht, E.**: Ztschr. f. klin. Med., **130**, 468, 1936. (15.) **Minot, G. R.,** and **Castle, W. B.**: Lancet, **2**, 319, 1935. (16.) **Morris, R. S., Schiff, L., Burger, G.,** and **Sherman, J. E.**: Am. J. Med. Sci., **184**, 778, 1932. (17.) **Parsons, L. G., Hickmans, E. M.,** and **Finch, E.**: Arch. Dis. Child., **12**, 369, 1937. (18.) **Reimann, F., Steiner, H.,** and **Grünfeld, M.**: Ztschr. f. klin. Med., **131**, 444, 1937. (19.) **Richter,**

O., Ivy, A. C., and **Meyer, A. F.**: Proc. Soc. Exp. Biol. and Med., **31**, 550, 1934. (**20.**) **Richter, O., Meyer, A. E.,** and **Ivy, A. C.**: Ann. Int. Med., **7**, 353, 1933. (**21.**) **Scheerer, H.**: Folia hæmatol., **56**, 321, 1937. (**22.**) **Seyfarth, C.**: Ibid., **34**, 7, 1927. (**23.**) **Singer, K.**: Klin. Wchnschr., **14**, 200, 1935. (**24.**) **Sturgis, C. C.,** and **Isaacs, R.**: J. Am. Med. Assn., **93**, 747, 1929. (**25.**) **Vaughan, J. M.,** and **Muller, G. L.**: J. Clin. Invest., **11**, 129, 1932. (**26.**) **Vaughan, J. M., Muller, G. L.,** and **Zetzel, L.**: Brit. J. Exp. Path., **11**, 456, 1930. (**27.**) **Wigodsky, H. S.** and **Ivy, A. C.**: Proc. Soc. Exp. Biol. and Med., **38**, 787, 1938. (**28.**) **Wigodsky, H. S., Richter, O.,** and **Ivy, A. C.**: Proc. Am. Physiol. Soc., **122**, 215, 1938. (**29.**) **Wilkinson, J. F.**: Brit. Med. J., **1**, 236, 1930. (**30.**) **Wintrobe, M. M.**: J. Lab. and Clin. Med., **17**, 899, 1932. (**31.**) **Wintrobe, M. M.,** and **Shumacker, H. B.**: J. Clin. Invest., **14**, 837, 1935. (**32.**) **Wintrobe, M. M., Kinsey, R. E., Blount, R. C.,** and **Trager, W.**: Am. J. Med. Sci., **193**, 449, 1937.

REPORT OF A CASE OF APLASTIC ANEMIA FOLLOWING GOLD INJECTIONS IN WHICH RECOVERY OCCURRED.

By Maxwell M. Wintrobe, M.D.,
associate in medicine, the johns hopkins university; assistant physician the johns hopkins hospital,

Averill Stowell, M.D.,
interne, the johns hopkins hospital, baltimore, md.,
and
Rufus M. Roll, M.D.,
interne, new york hospital,
new york,

(From the Department of Medicine, Johns Hopkins University and the Medical Clinic, Johns Hopkins Hospital).

Since the introduction of gold compounds for the treatment of tuberculosis, arthritis and lupus erythematosus, a number of reports have indicated that many hazards[6] attend this type of treatment. These include immediate reactions such as syncope, nausea, edema of the lips and face, headache, malaise, chills and fever; and delayed toxic effects. The latter may appear a few hours after the administration of gold or may not be observed for several weeks. They result from involvement of the skin, mucous membranes, kidneys, liver or other organs, as well as the hematopoietic system, and may be very serious in their consequences.

The blood changes which have been observed following the use of gold are similar to those which are now well recognized accidents associated with the use of the organic arsenicals. As with the latter, these hematopoietic effects are extremely rare in comparison to the number of times the drug is administered. In the summary by Dameshek[5] of cases reported until 1933, 1 case of purpura hemorrhagica, 1 of granulocytopenia, 5 of aplastic anemia, and 1 of "hypoplastic" anemia are included. From the discussion of these case reports it is evident that a few additional cases of similar character have been observed but have not been reported. Émile-Weil and Bousser[8] referred to 30 instances of "hemorrhagic accidents" following the use of gold but the exact nature of these reactions was not given.

A number of reports have appeared since Dameshek's review in 1933. Like those reported before 1933, these have come chiefly from France where gold treatment has attained its widest popularity. The cases are classed under a great variety of diagnoses but we have divided them into 3 groups in accordance with criteria which are now generally accepted; namely, "aplastic anemia" when red blood corpuscles, leukocytes of the myeloid series and platelets are diminished in number; "granulocytopenia," when only the leukocytes are affected; and "purpura hemorrhagica" when platelets are reduced in number and any accompanying anemia can be accounted for by blood loss resulting from hemorrhages. In such cases there may be leukocytosis. This classification does not deny the possibility that the three types of blood disorder represent different degrees of injury of the same fundamental character.

According to these criteria, 6 cases of aplastic anemia,[7,9,19,23,41,42] 9 cases of granulocytopenia,[3,4,12,14,28,32,40] and 6 of purpura hemorrhagica,[12,16,18,34,39] may be added to those listed by Dameshek. With the latter's case of aplastic anemia this makes a total of 13 cases of aplastic anemia, 10 of granulocytopenia, and 7 of purpura hemorrhagica, a total of 28 cases in all.

In 8 of the 10 cases of granulocytopenia and in 3 of the 7 cases of purpura which have developed following injections of gold, recovery occurred; but when the injury was such that anemia, granulocytopenia and thrombocytopenia developed, the result was usually fatal.

We have been able to find only 2 adequately described cases of aplastic anemia following gold therapy in which recovery occurred. Jullien's patient[19] received a total of 6 gm. of crisalbine during a period of 3 months for the treatment of pulmonary tuberculosis. Shortly afterwards the red cell count was found to be 2,380,000, the hemoglobin 65%, the leukocytes 1550 and 81% of the latter were of the lymphoid series. The platelet count was not recorded but bleeding from the nose and uterus, and retinal hemorrhages occurred. The erythrocyte count fell as low as 1,760,000; the leukocytes to 930. Blood transfusions and Roentgen ray therapy were given. Seven months after anemia was first observed, the erythrocyte count was 3,850,000, the hemoglobin 75% and the leukocytes 5890, of which only 49% were lymphocytes. The patient was symptomatically well.

In the case described by Weissenbach, *et al.*[42] it was not shown that the blood could be maintained at a satisfactory level without frequent repetition of blood transfusions. This patient had been given two courses of treatment for chronic infectious arthritis, receiving a total of 1.5 gm. of gold and sodium thiopropanol sulphonate in the first course and 2.0 gm. in the second, which was commenced after a rest period of 2 months. At the end of the second course a fleeting erythematous eruption appeared, weakness and purpura developed, and severe anemia of the aplastic type was

discovered. The erythrocyte count fell as low as 672,000 per c.mm. and the leukocytes numbered 800 per c.mm. Bleeding time was greatly prolonged. One year later, the patient was still alive, the erythrocyte count was 4,110,000 and the leukocytes ranged between 2000 and 5000 with 47 to 56% polymorphonuclears. The patient had received 50 blood transfusions and sodium nucleinate, pentnucleotide and liver extract had been used as well. In discussing Weissenbach's report, Rist referred to a patient of his own with aplastic anemia following gold therapy who had "recovered" but gave no details.

The following patient developed severe aplastic anemia while receiving gold injections and is alive and symptomatically well almost 4 years after the onset of anemia. Although her blood cannot be regarded as perfectly normal, she is able to maintain it at a satisfactory level even though she has had no blood transfusion nor other form of treatment for 2½ years. She had been given a total of 230 mg. gold and sodium thiosulphate. She received a total of 9 blood transfusions and 17 months after the onset of aplastic anemia hysterectomy was carried out with the object of removing this source of blood loss.

Case Report. L. G., U-53639, a white woman, aged 34, came to the Skin Clinic of this hospital because of an eruption on her nose and face. She had been seen in the gynecologic clinic 6 months before where the hemoglobin was found to be 92%, the leukocytes numbered 13,500 and there were 72% neutrophils. On examination a series of well defined infiltrated plaques, which were subacutely inflamed and covered by slightly adherent scales, were present on the face, extending from the right mastoid prominence across the bridge of the nose to the malar process on the left. Small lesions were present on the scalp, behind the right ear and on the chest. This was diagnosed as lupus erythematosus (subsequently confirmed by biopsy). On August 17, 1934, 10 mg. of gold sodium thiosulphate were injected intravenously. For the next 2 weeks the same dose was repeated each week, and on the fourth week, the dose was increased to 25 mg. weekly, this amount being given for 5 weeks. On October 11, 1934, following an increase of the dose to 50 mg., nausea and vomiting developed. By the next week slight dermatitis with marked pruritus was noted about the arms, especially on the flexor surfaces. The dose at this time was reduced to 25 mg. On October 24, there was anorexia and slight edema of the face especially about the eyelids, and it was noted that the eruption was more extensive. Severe diarrhea developed and the patient felt weak, ill and generally run down. Skin biopsy at this time showed acute disseminating lupus erythematosus. Gold sodium thiosulphate injections were immediately stopped. Weakness increased in severity, and about December 5, pruritus became more marked. Four days later, blood examination revealed: erythrocyte count 1,250,000, hemoglobin 5.3 gm., volume of packed red cells 15.7 cc., mean corpuscular volume 125 c.μ., leukocyte count 4200 per c.mm., and platelet count 46,000 per c.mm. Differential count: myelocytes 1%, juvenile neutrophils 4, segmented neutrophils 46, eosinophils 2, lymphocytes 46, and monocytes 1%. There was very marked anisocytosis with many macrocytes and some oval forms, slight poikilocytosis, and no achromia. The smears were practically devoid of platelets. The bleeding time was 7 minutes.

The patient was admitted to the hospital on December 19. T. 99.2°, P. 96, R. 20, B. P. 110/54. Many small brown macules all over the body and dark

pigmentation in the folds of the skin, were noted. There was marked pallor. Ophthalmoscopic examination revealed numerous flame-shaped hemorrhages in both fundi. No petechial hemorrhages were found on the skin. General examination, including neurological, was negative. Four transfusions of 500 cc. each, as well as pentnucleotide, and iron and ammonium citrate were given.

Definite improvement followed the transfusions. The erythrocytes increased in number and their mean size was reduced to normal, although the leukocyte and thrombocyte counts were little changed (Fig. 1). When she was discharged on January 26, 1935, there remained a mottled, brownish pigmentation of the arms, trunk and face. The erythrocyte count was 3,290,000, leukocytes 3200, platelets 120,000.

The patient was readmitted to the hospital on February 6, because profuse uterine bleeding had developed 17 days after the previous menstrual period. This ceased after one transfusion and the patient was discharged.

Menorrhagia recurred 1 month later. At this time the patient began to complain of a sensation of pins and needles in the limbs. Neurologic examination at this time and on many subsequent occasions failed to reveal changes in the nervous system other than a slight diminution of vibratory sense over the legs as high as the anterior superior iliac spines. Nevertheless, because of these symptoms and because repeated blood examinations revealed many macrocytes and a persistently high mean corpuscular volume, she was admitted to the hospital for the third time on June 13 for further study. Gastric analysis following the injection of histamine showed hydrochloric acid, free 22, combined 17. During the next month the patient was given 7 doses of liver extract (Lilly, 10 cc.). This therapy was associated with only a slight fluctuation in reticulocytes (from 0.75 to 2.90%) and the erythrocyte count did not change. The administration of sodium cacodylate (6 doses of 0.05 gm. intravenously) was associated with a similar fluctuation in reticulocytes. On July 19, 1935 a biopsy of the marrow of the tibia revealed fatty marrow for the most part, and the majority (84%) of the cells found there were lymphocytes. No nucleated red cells were seen (Table 1). Two more transfusions were given and the patient was discharged. Her condition had not been changed by liver therapy but following the transfusions the menorrhagia was less severe.

TABLE 1.—DIFFERENTIAL COUNTS OF BONE MARROW SMEARS.

	Sternum normal %	Tibia 7/19/35 %	Sternum 2/13/36 %	Sternum 6/15/38 %	Sternum 3/15/39 %
WBC					
Myeloblasts	2.0	..	3.0	0.3	1.0
Undiff. myelocytes	5.0	..	4.0	3.3	2.3
Diff. Myel. neutro.	12.0	..	8.0	21.0	23.3
eosin.	1.5	..	4.5	....	1.3
baso.	0.3				
Metamyelocytes neutro.	22.0	2	14.0	23.4	30.7
eosin.	....	..	2.0	....	0.7
PMN neutrophils	20.0	13	8.0	12.7	18.3
eosinophils	2.0	..	0.5	....	0.7
basophils	0.2				
Lymphocytes	10.0	84	42.5	24.3	11.0
Plasma cells	0.4	..	....	1.3	
Monocytes	2.0	1	1.5	2.0	1.0
Macrophages and reticulum cells	0.2	..	1.5	1.3	0.3
Megakaryocytes	0.4	..	....	0.4	
RBC					
Primitive erythroblasts and macroblasts	4.0	..	2.0	0.3	1.7
Normoblasts	18.0	..	8.0	9.7	7.7
Reticulocytes	5.0			5.2	1.5

Except for moderate weakness the patient had few complaints. On February 13, 1936, she was admitted for the fourth time and a sternal marrow biopsy was done. The marrow showed a few fatty aplastic areas interspersed among other areas of active hematopoiesis. The differential count of smears made from the marrow (Table 1) showed much more than the normal number of lymphocytes, with reduction in the neutrophilic myeloid cells, no megaloblasts, few macroblasts, and a few normoblasts.

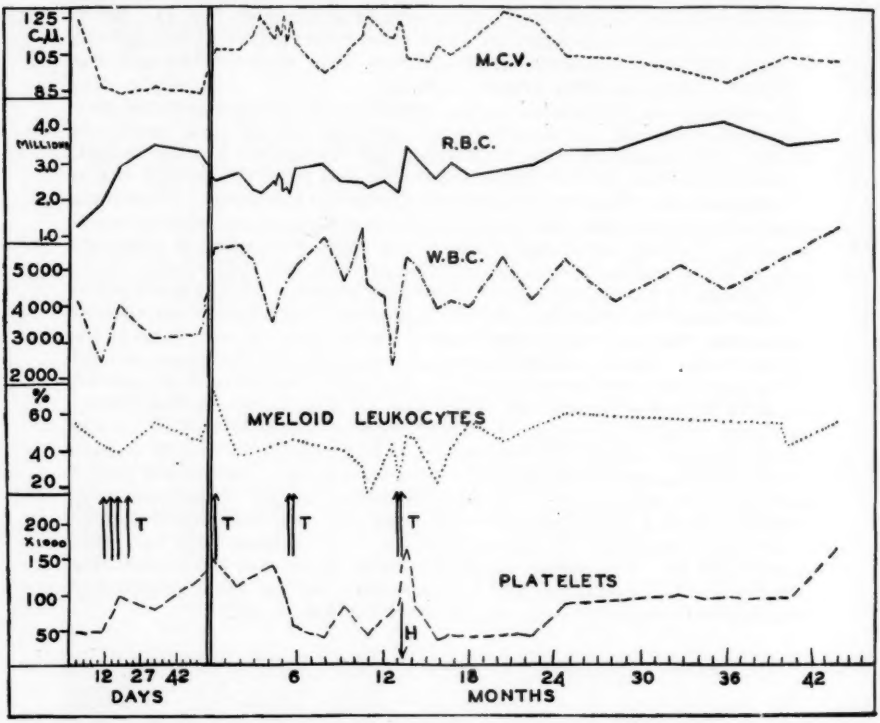

FIG. 1.—Changes in the blood of a case of aplastic anemia following the intravenous injection of gold sodium thiosulphate, the observations cover a period of 4 years. M. C. V. refers to mean corpuscular volume, R. B. C. to red cell count and W. B. C. to white cell count. The arrows pointing upwards and marked "T" indicate transfusions and the arrow pointing downwards and marked "H" indicates the time of hysterectomy.

Treatment of the anemia was attempted again. However, injections of a fetal liver extract, ascorbic acid, and commercial liver extract were followed by no significant blood changes. On March 6 the erythrocyte count was 2,200,000, hemoglobin 9.3 gm., volume of packed red cells 26.9 cc., mean corpuscular volume 122 c.μ., platelets 80,000, leukocytes 3800 and neutrophils 23%. Hysterectomy was then performed (March 13) with the object of removing this source of blood loss, even though menorrhagia was no longer very severe. The operation was borne without mishap. Two transfusions were given before operation. Six days after operation the erythrocyte count was 3,410,000, hemoglobin 12.0 gm., volume of packed red cells 34.9 cc., platelets 166,000, leukocytes 5350 with 43% segmented and juvenile neutrophils. She was discharged a week later in good condition.

Since March 11, 1936, no further blood transfusions have been given. During the next year the patient continued to be well, complaining only of slight weakness and occasional sensations of numbness and tingling in her fingers and occasional swaying or stumbling on walking. On March 3, 1937, some diminution in vibratory sense in the legs was noted but the neurologic examination was otherwise normal. A tourniquet test showed numerous fine petechiæ.

A sternal marrow puncture was done on June 15, 1938. The specimen was quite cellular and showed that the lymphocytosis observed 2 years before had almost disappeared, the percentage having dropped to 24, while the myeloid leukocytes were much more numerous than they had been (Table 1). Nucleated red corpuscles were still somewhat reduced in number. No megaloblasts were found. The erythrocyte count of the blood at this time was 3,580,000, the hemoglobin 12.6 gm., the volume of packed red cells 38.0 cc., the mean corpuscular volume 103 c.μ., and occasional macrocytes were found in the blood smear. The platelet count was 91,000, although bleeding time was only 5 minutes, and clot retraction was complete in 1 hour. Relative lymphocytosis (52%) was still present. Symptomatically, the patient was well and able to carry on her housework. Her only complaint was of occasional pain in the back which is considered to be of arthritic origin.

The most recent examination of the patient was made on September 28, 1938, almost 4 years after the onset of her anemia. In spite of the fact that no blood transfusions had been given for 2½ years, the erythrocyte count remained at 3,660,000, the hemoglobin 12.6 gm., and the leukocytes numbered 6200 of which only 41% were lymphocytes. It is noteworthy that macrocytosis was still present, as indicated by the mean corpuscular volume of 100 c.μ., and the presence of macrocytes in the blood smear. Platelets were moderately plentiful in the blood smear and numbered 157,000 by count. Bleeding time was 5 minutes, coagulation time 13 minutes and the clot retracted in 1½ hours.*

Discussion. *Relation of Gold Injections to Aplastic Anemia.* The evidence that the injection of gold compounds is the cause of the hematopoietic changes which occasionally follow its use is only circumstantial. In our own case, the possibility must be considered that the blood changes observed were caused by the disease for which gold treatment was given, namely lupus erythematosus. Few adequate blood studies in cases of this disease are available. The most common change in the blood, when one occurs at all, seems to be leukopenia.[27] One of Templeton's[37] patients, however, had aplastic anemia even though gold therapy was not given. Two others were reported by him as having thrombocytopenia but in one of these the blood studies were inadequate, while in the other tuberculosis of the spleen might have accounted for the blood changes noted.

Incrimination of gold preparations as a cause of hematopoietic changes is made more plausible by the fact that similar changes are sometimes found following the injection of organic arsenicals. As with the latter, it seems impossible to predict whether in any given case untoward effects on the hematopoietic system are likely to occur. There is no correlation between the amount of gold given

*On reëxamination on March 15, 1939, the blood findings were essentially the same except that the platelet count had risen still further and numerous platelets were found in the blood smear.

or the duration of the treatment and toxic effects. Rist, in discussing the case of Weissenbach, *et al.*,[42] referred to a patient who received 45 gm. of crysalbine (sodium and gold thiosulphate) with no apparent harmful results. Some form of hypersensitiveness is probably the underlying disorder.

Attempts to produce blood changes in animals by the injection of heavy metals have, with a few unconvincing exceptions, been unsuccessful. Émile-Weil and Bousser[8] produced retardation of coagulation of the blood of rabbits by the injection of Solganol, a gold salt. Bernard,[2] using crysalbine (gold and sodium thiosulphate) reported a 50% reduction of the platelet count and prolongation of bleeding time following injections into the femoral bone marrow of rats, but failed to produce changes by intravenous injections. Likewise Pan[31] and Heinild[15] failed to produce blood changes in rabbits by the injection of gold compounds and Shouse and Whipple[33] failed to do so by the injection of silver.

Our own attempts to produce blood changes in rabbits* by the intravenous injection of gold sodium thiosulphate have also been unsuccessful. One animal received four injections of 10 mg. each, another one injection of 50 mg., and a third nine injections of 50 mg. each over a period of 3 months. In the last 2 animals a slight reduction in the erythrocyte count followed the first injections of gold but this did not continue after subsequent injections, and no changes occurred in leukocytes or platelets.

Prognosis in Aplastic Anemia. Recovery following aplastic anemia is extremely unusual. Among the reported cases of "idiopathic" aplastic anemia, we have found an account of only 6 patients who lived more than a year after the onset of the anemia and even these cannot be considered as having completely recovered. Gibson's[10] patient, a girl aged 11 years, received daily injections of adrenalin for 6 years and remained symptomatically well although moderate anemia and leukopenia persisted. She died at the end of this time of hemorrhagic chicken-pox. Upham and Nelson[38] reported a case in which fetal liver feeding was associated with such improvement that the patient was still alive 2 years after the onset of the anemia. It is noteworthy that at the end of that time thrombocytopenia and moderate anemia were still present. Harrison's[11] patient was treated with 290 transfusions and died after 9 years of a delayed transfusion reaction.[21] This man was found to have hemochromatosis. A patient of Osato *et al.*[30] (Case VIII), after repeated blood transfusions and radiation of the long bones, was no longer anemic 22 months after anemia was first observed, but moderate leukopenia and thrombocytopenia still persisted. Another patient (Case VIII) showed moderate anemia, leukopenia and thrombocytopenia after 1 year. Atwood's patient,[1] a boy aged 12 years, whose red cell count fell as low as 970,000, after only 2 blood transfusions and administration of adrenalin and ephedrine, was found

* Aided by funds from a Parke, Davis & Co. grant for studies in hematology.

to be quite normal at the end of a year, with a leukocyte count of 4350 as the only indication of hematopoietic deficiency.

In cases of aplastic anemia following injection of the arsphenamines, prognosis is slightly more favorable. Of the 34 cases collected by McCarthy and Wilson[26] 6 (18%) were said to have recovered. Two of these cases (1 and 4), however, did not show a completely typical picture of aplastic anemia and one of them (Case 5) was still very anemic at the time of the report. Thus the proportion of recovered cases is reduced possibly to 9 or 10%. It is very difficult to discover what is the true mortality in cases of post-arsenical aplastic anemia from examination of case reports because cases in which recovery occurred are more likely to be reported than fatalities. Kadin[20] recently collected 17 cases of aplastic anemia following the use of arsenicals and added 3 of his own. This series includes 6 patients who made partial[22,25,27] or complete[17,35,36] recovery. Hawkins[13] reported a case in which recovery occurred and Lieberson and Weiss[24] have lately reported 2 more cases. Thus a total of about 13 patients have been reported as making complete or partial recovery following aplastic anemia caused by the injection of one of the organic arsenicals. One of the patients admitted to this hospital with post-arsenical aplastic anemia has improved.

The rarity of recovery in cases of aplastic anemia following gold injections has already been mentioned. Although the patient we have described is now symptomatically well 4 years following the onset of anemia, her blood is not entirely normal. The persisting macrocytosis is of considerable interest. With the failure of intensive liver therapy to affect the blood and the lack of achlorhydria, an Addisonian type of anemia can be ruled out. Elsewhere[43] one of us pointed out that macrocytosis may be associated with extramedullary blood formation. One wonders whether the development extra-medullary blood forming tissue may account not only for the macrocytosis in this case but also in part for the patient's recovery.

Summary. 1. A case of aplastic anemia, presumably due to the intravenous injection of a gold compound, is reported.

2. This patient is still alive 4 years after the development of aplastic anemia and is symptomatically well although the blood is not entirely normal. She has had no transfusions for 3 years.

3. The case reports of harmful hematopoietic effects following the use of gold are briefly reviewed, especially from the standpoint of prognosis. The reported case is the third of a total of 13 cases of aplastic anemia in which death had not occurred at the time of the report and is the second one which has attained almost complete recovery.

4. Experiments are mentioned in which attempts to produce changes in the blood of rabbits by the intravenous injection of gold failed. The evidence incriminating gold as a cause of blood changes is entirely circumstantial.

5. The literature dealing with recovery in cases of idiopathic

aplastic anemia and in cases following the injection of organic arsenicals, is reviewed. Only 6 of the former type have survived a year or longer while in the post-arsenical group 13 partial or complete recoveries have been recorded.

REFERENCES.

(1.) **Atwood, E. B.**: Canad. Med. Assn. J., **34**, 501, 1936. (2.) **Bernard, J.**: Sang, **9**, 85, 1935. (3.) **Braillon, J.**: Ibid., **8**, 99, 1934. (4.) **Chabaud, J., Ginsbourg, B.**, and **Langlet, L.**: Bull. et mém. Soc. méd. d. hôp. de. Paris, **27**, 1238, 1933. (5.) **Dameshek, W.**: New England J. Med., **210**, 687, 1934. (6.) **Driver, J. R.**, and **Weller, J. N.**: Arch. Dermat. and Syph., **23**, 87, 1931. (7.) **Ellman, P.**, and **Lawrence, J. S.**: Brit. Med. J., **2**, 622, 1935. (8.) **Émile-Weil, P.**, and **Bousser, J.**: Sang, **6**, 825, 1932. (9.) **Gautier, C., Seidmann, P.**, and **Baudouin, A.**: Bull. et mém. Soc. méd. d. hôp. de Paris, **24**, 1194, 1936. (10.) **Gibson, A. G.**: Lancet, **2**, 948, 1926. (11.) **Harrison, R. S.**: Guy's Hosp. Rep., **81**, 215, 1931. (12.) **Hartfall, S. J.**, and **Garland, H. G.**: Lancet, **2**, 8, 1935. (13.) **Hawkins, H. F.**: Texas State J. Med., **28**, 822, 1933. (14.) **Hedfeldt, A.**: Nord. med. Tidskr., **11**, 874, 1936. (15.) **Heinild, S.**: Acta med. Scandinav., **93**, 308, 1937. (16.) **Hudson, E. H.**: Lancet, **2**, 74, 1935. (17.) **Imrie, A. H.**: Ibid., p. 73. (18.) **Jones, H. W., Tocantin, L. M.**, and **Corson, E. F.**: Penna. Med. J., **37**, 809, 1934. (19.) **Jullien, W.**: Bull. et mém. Soc. méd. d. hôp. de Paris, **50**, 174, 1934. (20.) **Kadin, M.**: Arch. Dermat. and Syph., **37**, 787, 1938. (21.) **Kark, R. M.**: Guy's Hosp. Rep., **87**, 343, 1937.

(22.) **Knott, F. A.**: Guy's Hosp. Rep., **84**, 32, 1934. (23.) **Lavergne, V. de**, and **Bichat, J.**: Rev. méd. de l'est., **63**, 457, 1935. (24.) **Lieberson, A.**, and **Weiss, A.**: Ann. Int. Med., **10**, 1775, 1937. (25.) **Loveman, A. B.**: Ibid., **5**, 1238, 1932. (26.) **McCarthy, F. P.**, and **Wilson, R., Jr.**: J. Am. Med. Assn., **99**, 1557, 1932. (27.) **Madden, J. F.**: Arch. Derm. and Syph., **25**, 854, 1932. (28.) **Margarot, J., Rimbaud, P.**, and **Bétouliéres, P.**: Bull. Soc. franc. de derm. et syph., **41**, 545, 1934. (29.) **Merkelbach, O.**: Schweiz. med. Wchnschr., **63**, 546, 1933. (30.) **Osato, S., Hashimoto, T.**, and **Takigawa, T.**: Folia hæmatol., **53**, 42, 1934. (31.) **Pan, C. S.**: Dermat. Wchnschr., **102**, 648, 1936. (32.) **Rheinheimer, E. W.**, and **Smith, L. M.**: Southwest. Med., **17**, 239, 1933. (33.) **Shouse, S. S.**, and **Whipple, G. H.**: J. Exp. Med., **53**, 413, 1931. (34.) **Slat, W. J. B.**, and **Winkler, K. C.**: Nederl. tijdschr. v. Geneesk., **80**, 232, 1936. (35.) **Smith, D. L., Jr.**, and **Lyon, R. A.**: J. Pediat., **6**, 624, 1935. (36.) **Stephens, D. J.**: Am. J. Syph. and Neurol., **18**, 24, 1934. (37.) **Templeton, H. J.**: Arch. Dermat. and Syph., **29**, 700, 1933. (38.) **Upham, J. H.**, and **Nelson, G. I.**: Missouri State Med. Assn. J., **27**, 1, 1930. (39.) **Urgoiti, A.**, and **Hermida, J.**: Arch. d. med. cir. y especialid., **38**, 117, 1935. (40.) **Wechsler, L.**: Wien. klin. Wchnschr., **50**, 1360, 1937. (41.) **Weil, M. P., Oumansky, V.**, and **Langlois, L.**: Ann. de méd., **44**, 78, 1938. (42.) **Weissenbach, R. J., Martineau, J., Brocard, J.**, and **Malinsky, A.**: Bull. et mém. Soc. méd. d. hôp. de Paris, **22**, 1071, 1936. (43.) **Wintrobe, M. M.**: Arch. Int. Med., **57**, 289, 1936.

PHAGOCYTIC ACTIVITY IN LEUKEMIA.*

By Nell Hirschberg, Ph.D.,
CHICAGO, ILLINOIS.

(From the Department of Bacteriology, Cook County Hospital, and the Department of Bacteriology and Public Health, University of Illinois College of Medicine.)

The question of the infectious nature of leukemia has been discussed for many years. Because the various clinical manifestations of this disease often resemble an acute infectious process, it was assumed, for many years, that it might possibly have an infectious basis. Ordway[4] has made an excellent summary of the work in this field. Pagniez[5] reviewed the recent experimental work and concluded that leukemia was a disease of an infectious nature.

* This investigation was begun under the direction of the late Dr. Richard H. Jaffe.

The mature white cells in many forms of leukemia are apparently perfectly normal and should form the usual defense against infection. One might draw the conclusion that leukemia would probably have an infectious basis if the white cells are functioning in a normal manner. If the infection attacks the blood forming organs, then leukemia may result, and the few remaining normal mature cells are powerless to combat the overwhelming infection. If the mature cells which are present in leukemia are not functioning normally, they will not offer the usual defense against infection, the body will be exceedingly susceptible to infection and the usual infection associated with leukemia may be assumed to be superimposed upon an already weakened system. Howell[3] has shown that leukemic individuals may fail to develop antibodies after infection or vaccination, probably as a result of the marked changes in the hematopoietic tissues found in leukemia.

This study of various types of leukemia was undertaken to shed some light on that problem. An attempt was made to determine the condition of the mature cells remaining in the leukemia patient and circulating in the peripheral blood stream. Strumia and Boerner[7] have shown that the phagocytic activity of granulocytes increases proportionately with their age. Furthermore, this phagocytic power of white cells is a fair measure of their activity against bacteriologic infection. It was, therefore, decided to determine the phagocytic power of the mature white cells in the circulating blood of leukemic patients. In addition to this, complete bacteriologic studies of each patient were made, a rather complete history obtained, and the sedimentation rate of the red blood corpuscles was noted.

Bonanno[1] incubated 1 cc. of blood and 1 cc. of sodium citrate with a staphylococcus strain for 45 minutes at 37° C. He then made smears which he stained with methylene blue. He investigated 5 cases of chronic myeloid leukemia, 2 cases of chronic lymphatic leukemia and 1 case of hemocytoblastica. In his series of cases he found the phagocytic power was greatly reduced.

The present series consisted of 1 case of acute monocytic leukemia, 1 of acute aleukemic stem-cell leukemia, 3 of acute stem-cell leukemia, 3 of acute myelogenous leukemia, 2 of acute lymphatic leukemia, 1 of subacute aleukemic stem-cell leukemia, 5 of chronic myelogenous leukemia, and 4 of chronic lymphatic leukemia, a total of 20 cases. In addition, the blood of 20 individuals free from leukemia or infection was used as controls and the figures averaged.

The strain of staphylococcus used was readily phagocytized by normal blood but retained sufficient virulence so that only an average of 96% of the neutrophils of normal blood phagocytized the bacteria. The method of Strumia and Boerner[7] was used to set up the test. Two-tenths of 1 cc. of a washed suspension of white cells was mixed with 0.1 cc. of the staphylococcus suspension (containing approximately 1 billion bacteria per cc.) and 0.1 cc. of a 1 to 10 dilution of fresh normal pooled serum. The mixture was shaken for

30 minutes. At the end of that time smears were made, stained with Wright stain and examined. The number of cells which showed phagocytic activity was noted. Also, the kind of cell and the number of bacteria per cell were counted. Because in many cases the number of mature neutrophils was very small, the figures are only accurate when compared with this number.

Bacteriologic studies were made on nose, throat, blood, urine and stool cultures and on any other material from the patient which seemed to be from an infected source. Anærobic and ærobic cultures were made.

A simple sedimentation test was done, using 2 parts of 2% sodium citrate to 8 parts of blood. (According to Cutler *et al.*,[2] simple sedimentation tests are not satisfactory. However, it was felt that for purposes of comparison a simple sedimentation test was sufficient, inasmuch as the same method was used throughout, and the same sources of error were present in each study.) The blood-citrate mixture was drawn into a tuberculin syringe, placed upright on a stand and the drop noted in time intervals of 1, 5, 10, 15, 30, 45, 60, 90 and 120 minutes, and after 18 to 24 hours. This, of course, gives only comparative sedimentation rates.

Results (Table 1). In no case did more than 70% of the mature leukocytes phagocytize the bacteria. When this figure is contrasted with the average of 96% for the controls, it will be readily seen that the phagocytic power of the leukemic blood was decreased. In certain cases only 30% of the leukocytes were active. The average number of bacteria per cell varied from 38.2 to 2.6 bacteria, as contrasted with 18 for the normals.

With few exceptions, bacteriologic findings seemed to be positive in the acute leukemias. That is, the individuals showed a predominance of one organism in all the cultures either with a positive blood culture, indicating sepsis, or without positive blood culture.

The acute monocytic leukemia of 3 weeks' duration showed 7% mature neutrophils; 64% of these showed phagocytic activity with an average of 6.6 bacteria per cell. The sedimentation rate was very fast and dropped suddenly after the first few minutes. The patient had an almost pure culture of *Strep. hemolyticus* in the throat.

The acute aleukemic stem-cell leukemia of 1 week's duration showed 67% normal mature neutrophils, 50% of which showed phagocytic activity with an average of 20 bacteria per cell. The sedimentation rate began to fall very slowly and then took a sudden drop. This patient showed anærobic *Strep. hemolyticus* in the blood cultures.

The subacute aleukemic stem-cell leukemia of 8 months' duration showed 34% mature neutrophils, 62% of which showed phagocytic activity with an average of 7 bacteria per cell. The sedimentation rate was normal and the bacteriologic studies negative.

Three cases of acute stem-cell leukemia, varying from 1 to 8 months' duration, showed varying numbers of mature neutrophils and the percentage of those which showed phagocytic activity varied

from 20 to 70%. The number of bacteria per cell varied from 8 to 22.6. Two of these bloods showed a slightly increased sedimentation rate in the first few minutes, followed by a sudden drop. The third fell very fast in the beginning, slowed up after 15 minutes and then took a sudden drop. In 1 of these cases *Strep. viridans* predominated in all the cultures; in another there was a *Strep. hemolyticus* blood culture; in the third, bacteriologic study was negative.

TABLE 1.—RESULTS OF STUDIES IN LEUKEMIC PATIENTS.

Age (yrs.).	Sex.	Race.	Duration.	Type of leukemia.	Red blood cells in millions.	White blood cells in thousands.	Mature neutrophils (per cent).	Phagocytes (per cent).	Average No. bacteria per cell.	Sedimentation rate.	Bacteria present.
24	F	W	3 wks.	Acute monocytic	3.4	105.7	7	64	6.6	Very fast and sudden drop	*Str. Hemolyticus* in throat.
49	M	W	1 wk.	Acute aleukemic stem-cell	1.2	29.2	67	50	20	Slow and then sudden	Anerobic *Str. hemolyticus* in blood culture.
51	M	W	8 mos.	Subacute aleukemic stem-cell	3.9	8.6	34	64	7	Normal	Negative.
40	F	N	8 mos.	Acute stem-cell	3.3	170	10	70	8.7	Very fast and sudden drop	*Str. viridans* predominant in all cultures.
57	M	W	4 wks.	Acute stem-cell	2.5	279.6	1+	5 polys counted 1 phagocyte (20%)	8	Slow and then sudden drop	*Str. hemolyticus* in blood culture and all other cultures.
16	M	W	1 mo.	Acute stem-cell	1.1	4.3	20	56	22.6	Slow then very sudden	Negative.
15	F	W	1 wk.	Acute myelogenous	1.1	272	9	43	3.7	Slow—fast, large drops, slow	*Str. viridans* in blood and all other cultures Vincent's angina.
51	F	W	2 wks.	Acute myelogenous	1.3	104	15	40	10	Very slow, average drops	*Sta. albus.* in blood and all other cultures
23	F	N	3 mos.	Acute myelogenous	1.2	38.6	5	40	5.3	Slow, very sudden and large drop—slow	*B. mucosus* in all cultures.
23	M	W	2 yrs.	Chronic myelogenous	4.5	50.5	70	54	4	Normal	*Str. viridans* in blood culture and all other cultures.
40	M	W	2yrs.	Chronic myelogenous	1.2	96	20	44	6.4	Normal (slow)	Negative.
51	F	N	2 yrs.	Chronic myelogenous	2.8	560	37	60	7.5	Normal (fast)	Negative.
33	F	W	?	Chronic myelogenous	2.3	52.8	10	47	6	Normal	Negative.
49	F	N	3 mos.	Chronic myelogenous	4.6	350	27	37	4.6	Very slow	*Str. viridans* in blood and ascites and all other cultures.
40	M	W	9 mos.	Acute lymphatic	1.3	8.6	23	70	38.2	Sudden drop slow.	Negative.
6	M	N	3 wks.	Acute lymphatic	3.2	96.7	28	50	3	Normal	Negative.
57	M	W	1 yr.	Chronic lymphatic	3.1	83.8	52	30	3	Normal (fast)	*Str. hemolyticus* in blood and all other cultures.
55	M	W	3 yrs.	Chronic lymphatic	3.2	120	63	38	2.6	Normal (slow)	Negative.
65	M	W	6 mos.	Chronic lymphatic	2.9	14	35	51	22.19	Normal	Negative.
62	M	N	4 mos.	Chronic lymphatic	3.4	81.5	2	34	14.3	Normal	Negative.

The acute myelogenous leukemias of from 1 week to 3 months' duration showed from 5 to 15% mature neutrophils. The number of neutrophils showing phagocytic activity was around 40% in all 3 cases. The number of bacteria per cell varied from 3.7 to 10. The sedimentation rate was slow at the outset, followed by a fast, sudden and large drop and then a slow decrease. Each of the cases showed positive bacteriologic findings. In one of them, *Strep. viridans* was found in the blood culture as well as in all the other cultures and an acute Vincent's angina was present. The second patient showed *Staph. albus* in the blood culture and this organism predominated in all other cultures. In the third patient, Friedländer's bacillus predominated in all the cultures.

The 5 cases of chronic myelogenous leukemia varied from 3 months to 2 years' duration. The percentage of mature neutrophils varied from 10 to 70 and the phagocytic activity of these cells ranged from 37 to 60%. The average number of bacteria per cell varied from 4 to 7.5, a very small range. The sedimentation rate was normal in all 4 cases. One of these 4 showed *Strep. viridans* in a blood culture and predominating in all the other cultures. The fifth case showed a very fast sedimentation rate and *Strep. viridans* was isolated from all of the cultures, including the blood cultures and ascitic fluid cultures.

Two cases of acute lymphatic leukemia, one of 3 weeks' duration, showed approximately the same number of mature neutrophils: 28 and 23% respectively. Fifty per cent of the cells of the case of short duration showed phagocytic activity, with an average of 3 bacteria per cell. This was in marked contrast to the 70% of mature neutrophils in the case of longer duration which showed an average of 38.2 bacteria per cell. The sedimentation rate of this latter case dropped suddenly and then decreased slowly. The bacteriologic study in both cases was negative.

Four cases of chronic lymphatic leukemia, from 4 months to 3 years' duration, had from 30 to 51% of mature neutrophils showing phagocytic activity. The average number of bacteria per cell ranged from 3 to 22.19. The sedimentation rate in all 4 cases was normal, although one patient in a terminal state showed a very slightly increased rate. This patient also showed *Strep. hemolyticus* in the blood culture and other cultures. The bacteriologic findings in the other cases were negative.

These figures bring to light several very interesting facts. First of all they show that the sedimentation rate varies, as one would expect, with the duration and severity of the illness. In addition, those leukemic patients who seemed to be running a fairly slow, chronic course were, for the most part, free from systemic infection.

It is obvious that the percentage of mature neutrophils which showed phagocytic activity is considerably lower than normal. The average number of bacteria per cell was also lower than normal. It should be mentioned here that a few of the immature neutrophils phagocytized bacteria. This was especially marked in the acute

monocytic leukemia, but, for purposes of comparison, these figures were eliminated from the results. Strumia and Boerner[7] have made an excellent study of the phagocytic activity of immature leukocytes.

Discussion. In his book on the sedimentation rate of red blood corpuscles, Reichel[6] describes the factors which may influence this rate. Most of them were eliminated in this study. Since no attempt was made to secure an accurate sedimentation rate, all of the rates obtained are comparable. Every test was done in the same tuberculin syringe at room temperature (20° C.) within one-half hour after the blood was drawn. Reichel[6] found an increased sedimentation rate in leukemia. It is, therefore, interesting to note that many of the chronic cases which were investigated in this study showed normal rates.

Concerning the bacteriologic studies, it appears that the chronic cases were quite free from severe bacterial infection. Perhaps that is the reason they are chronic cases. However, it seems more probable that because they are chronic they are more resistant to infection. All of the chronic cases showed a fairly high phagocytic activity. In those cases with positive bacteriologic findings it appears that the infection is superimposed upon the leukemia, possibly due to the decreased phagocytic activity.

It would seem, therefore, that leukemia is not an infectious disease; that the infection so often found in leukemia is superimposed upon that condition and may be due, in part, to the decreased resistance of the leukemic individuals, as evidenced by the decrease in the phagocytic activity of their blood.

Summary. 1. Phagocytic studies, sedimentation rates, and bacteriologic studies were made in 20 cases of various types of leukemia.

2. The number of mature neutrophils in the peripheral blood of leukemic individuals which showed phagocytic activity was greatly decreased from normal and the phagocytic activity of the existing cells was also greatly decreased.

3. The sedimentation rate was increased in most of the acute cases but was normal in most of the chronic cases of leukemia.

4. Significant bacteriologic findings were noted in most of the acute cases.

5. It seems probable that leukemia is not itself an infectious disease but that infection so often found associated with that disease iss uperimposed upon the leukemia.

6. This may be due to the decreased resistance of the leukemic individual, as evidenced by the decrease in phagocytic activity of the mature neutrophils.

REFERENCES.

(1.) **Bonanno, A. M.**: Boll. d'Istituto Sieroterap., **16**, 40, 1937. (2.) **Cutler, J. W., Park, F. R.**, and **Herr, B. S.**: Am. J. Med. Sci., **195**, 734, 1938. (3.) **Howell, K. M.**: Arch. Int. Med., **26**, 706, 1920. (4.) **Ordway, T., Gorham, L. W.**, and **Beebe, R. T.**: Leukemia, Oxford Medicine, New York, Oxford University Press, II/3, Chap. 17, 1936. (5.) **Pagniez, P.**: Presse médicale, **44**, 1072, 1936. (6.) **Reichel, H.**: Blutkörperchensenkung, Vienna, Julius Springer, 1936. (7.) **Strumia, M. M.**, and **Boerner, F.**: Am. J. Path., **13**, 335, 1937.

BOOK REVIEWS AND NOTICES.

MODERN SURGICAL TECHNIC. In 3 Volumes. Vol. 1. General Operative Considerations: Surgery of the Head and Neck, and Plastic Surgery. Vol. 2. Surgery of the Nerves, Vessels, Bone, Breast and Chest; Vol. 3. Abdominal Surgery, Hernia, Genito-Urinary and Gynecologic Surgery. By MAX THOREK, M.D., K.L.H. (FRANCE), K.C. (ITALY), Professor of Clinical Surgery, Cook County Graduate School of Medicine; Attending Surgeon, Cook County Hospital; Surgeon-in-Chief, The American Hospital, etc. With a Foreword by DONALD C. BALFOUR, M.B., M.D. (TOR.), LL.D., F.A.C.S., F.R.A.C.S., Head of Section in Division of Surgery, The Mayo Clinic; Director and Professor of Surgery, The Mayo Foundation for Medical Education and Research, Graduate School, University of Minnesota, etc. Pp. 2045; 2174 illustrations, originals principally by W. C. Shepard. Philadelphia: J. B. Lippincott Company, 1938. Price, $33 per set.

THE author has attempted to write an operative surgery intermediate in length between the long system and the single volume surgery. The book aims to contain enough surgical technique and a sufficiently detailed description of each commonly performed operation to be used by students and general surgeons and those general practitioners who are occasionally called upon to perform emergency operations. He has included sufficient anatomic details to acquaint the reader with the operative field, and he has used the step-by-step method in describing the operation.

Volume 1 is divided into two parts. In Part I. General Operative Considerations, the author describes the general relations of the surgeon to his art and to his patient and discusses in a relatively brief manner the set-up of the operating room, sterilization of the surgical supplies and the various types of anesthesia. His descriptions are those of his own technique in most part, and these probably are not always acceptable to the reader. For instance, the author illustrates and lays some stress upon the sealing of the umbilical pit with collodion in preparation for operation. This is not a procedure which is generally accepted in most clinics as being very valuable. His sections on the preoperative and postoperative complications are relatively brief.

In the chapter on anesthesia, the author unfortunately does not mention any of the newer anesthetic agents, such as vinethene and cyclopropane. He is not convinced that any of the spinal anesthetics, except novocaine, are of particular value. His section on regional anesthesia is brief but to the point.

Part II of Volume 1 is concerned with the surgery of the head and neck and plastic surgery. A relatively long chapter on the surgery of the skull and brain describes various surgical procedures in detail. This chapter is well illustrated and would be extremely useful for one who occasionally has to perform operations on this part of the body. The author surprisingly does not mention the use of 50% glucose intravenously in the control of increased intracranial tension, although he considers the use of hypertonic saline, magnesium sulphate and spinal puncture. The chapters on surgery of the ears and tonsils are well written and profusely illustrated. A considerable portion of one chapter is given over to a description of neurectomy of several facial nerves. It would appear that disproportionate space is given to this minor subject. The final chapters of the book include

descriptions of the surgery of the salivary glands, jaws, orbit and eye, and the surgery of the nose, neck and cervical endocrine glands. A brief and well-illustrated chapter on the general principles of plastic surgery finishes the first volume.

Volume 2: This volume is likewise divided into two parts. The first part is concerned with the surgery of the nerves, vessels and bones. In the chapter on peripheral nerves, such operations as neurolysis, anastomosis and operations for spastic paralysis are described and illustrated. The chapter on the surgery of the sympathetic nervous system is a mixture of old and new. The author describes the various operations which were advocated during the first years of sympathetic surgery, periarterial sympathectomy and so-called cervicothoracic sympathectomy. In discussing the latter operation, he does not mention the resection of the first, second and third thoracic ganglia which have been shown in recent publications to be the important sympathetic structures to be removed in sympathectomy of the upper extremities. He does not describe the Gask-Telford operation for anterior thoracic sympathectomy, nor the White-Smithwick or Adson technique for posterior sympathectomy. Included in this section are such operations as chordotomy and adrenal denervation as practised by Crile.

In the chapter on surgery of the vascular system, the operator gives well-illustrated sections on ligations of the arteries. His section on surgery of veins, however, does not include the more modern ideas concerning ligation of the saphenous vein. His section on aneurysms is good. He gives in great detail a discussion on blood transfusions with illustrations of innumerable types of transfusion apparatus.

The chapter on orthopedic surgery describes almost all of the commonly performed operations. Very little is mentioned about the subdeltoid bursa (here Codman's name is spelled Cadman). The author includes in this chapter, strangely enough, operations for meningocele and pilonidal sinus. The chapter on amputation is well written and very nicely illustrated. A rather long chapter on fractures and dislocations considers mostly the operative treatment of these lesions. Little is said concerning the more modern treatment of fractures with the use of various mechanical appliances for reduction. In discussing recurrent dislocations of the shoulder the author merely mentions the Nicola operation, although he describes in detail other operations which are less generally used. The last part of the volume concerns the surgery of the breast and chest. Here the author includes a fairly large section on his own methods of plastic surgery of the breast. A fairly complete outline of the various commonly accepted operations on the chest are described and well illustrated. The part is concluded by a chapter on the heart and pericardium, in which there is a good summary of the work of Claude Beck.

Volume 3, concerned with the surgery of the abdomen, is divided into two parts. First is the surgery of the abdomen and the abdominal organs and, second, surgery of the pelvic region. The first part includes a discussion of the various commonly used procedures in dealing with diseases of the stomach, intestines, liver, pancreas, spleen and hernia. These sections are well written and the author is evidently writing from a mass of clinical experience. He has illustrated the text profusely and in most cases with diagrammatic illustrations which increase the clarity of the text. The chapter on the surgery of the pelvic regions concerns the commoner gynecologic operations in a relatively brief manner, and perhaps more fully discusses the operations upon the kidney, bladder, prostate and external genitalia.

In reviewing these 3 volumes, one is impressed by the mass of material which is included in them, and is not surprised that there may be some

things left out which would appear important to individual readers. On the whole, the book serves a very useful purpose for those who are interested in obtaining a rapid and concise review of the methods of performing various surgical operations. The text is concisely written and perhaps a little too rigidly adheres to the step-by-step method, although the advantage of this type of text for beginners is evident. The illustrations as a whole are well done. The author has included in his list more operations proposed and performed by European surgeons than would seem justifiable. By the same token, he has omitted a considerable amount of original work which can be found in the American literature.

The Reviewer is glad to have these volumes to add to his library and he is certain to use them frequently. They should be a part of the library of most operating surgeons and especially of those who occasionally operate and wish to have an authoritative volume to which they may turn for rapid reference. L. F.

OUT OF THE RUNNING. By G. GERTRUDE HOOPES. With a Foreword by EDGAR A. DOLL, PH.D., Director of the Department of Research, The Training School at Vineland, N. J., and with Clinical Notes by WINTHROP M. PHELPS, M.D., Director of the Children's Rehabitation Institute, Inc., Baltimore. Pp. 158; illustrated. Springfield, Ill.: Charles C Thomas, 1939. Price, $2.00.

STEVENSON'S tuberculosis, Ibsen's diabetes, Sternberg's periodic insanity, and Helen Keller's twofold afflictions did not prevent their rise to fame. Indeed, art has been said to be the offspring of poor health. The author was badly crippled at birth and is now fifty-eight years old. At the earnest solicitation of friends, she reveals her life in an autobiography of two parts, the first of which was written twenty years ago. It appears probable that at birth, intracranial injury caused hemorrhage into the basal nuclei, which brought about great, permanent, physical disability. From this resulted disabled locomotion, athetoid movements of the upper extremities, disturbance in the mechanism of breathing, and imperfect movements of tongue rendering oral speech impossible. Since a fall in early childhood, locomotion has been possible only through the use of an especially constructed tricycle. The frequent involuntary movements are so disabling as to permit of only one-finger typing, and verbal communication is through the medium of her shorthand manual sign language, somewhat similar to that of deaf mutes; conversation is accomplished by pointing to the letters of the alphabet attached to a piece of cloth. With such limited means of motor expression, these achievements compel admiration from all familiar with her. Many afflicted by severe physical handicaps will gain courage from reading the excellently written life by this mentally superior woman, who manifests neither self-pity nor craving for sympathy.

N. Y.

LIFE'S BEGINNING ON THE EARTH. By R. BEUTNER, M.D., PH.D., Professor of Pharmacology at the Hahnemann Medical College and Hospital of Philadelphia. Pp. 222; 80 illustrations. Baltimore: The Williams & Wilkins Company, 1938. Price, $3.00.

THE past few years have witnessed much literary popularization of the results of scientific investigation. The justification for the widespread dissemination of such knowledge is a debatable question, as even with accurate descriptions of experiments and careful interpretations of their meaning by the author, the layman, for whom such works are written, is in constant danger of obtaining erroneous conceptions because of his own

lack of critical judgment in such matters. Much as a great poem loses its meaning and force when put into the verbal and grammatical construction of every-day conversational speech, so do the implications of a scientific treatise become distorted when couched in other than scientific language. Such books are sold to the public upon the basis of their "startling revelations" and by their appeal to the mystical, the excitingly romantic attitudes which in a sober science do not exist.

In *Life's Beginning on Earth* the author attempts to convince his audience of the truth of his hypothesis by four "approaches": 1, The resemblances between vital growth and crystallization; 2, an interpretation of life as the outstanding property of the carbon atom; 3, the importance of salt and water for life; and 4, the animal considered as a machine.

As usual in such pseudoscientific literature, the author is not careful to distinguish emphatically between fact and theory. His whole-hearted acceptance of the viability of viruses, for example, differs with the attitudes of the very scientists whose work he adopts as evidence.

The book abounds in colorful descriptions such as: "On the hot and sultry earth, loaded with lifeless organic matter, violent thunderstorms raged; unspeakably brilliant and powerful lightnings played in the heavens, loosening frightful forces upon the carbon-containing gases of the atmosphere, bringing into existence numerous compounds of carbon. After millions of years self-regenerating enzymes were formed"; this is the essence of the author's hypothesis.

Life's Beginning on Earth is an entertainingly fanciful story which lacks in importance the wealth of its author's enthusiasm.

D. C.

CLINICAL LABORATORY METHODS AND DIAGNOSIS. A Textbook on Laboratory Procedures with Their Interpretation. By R. B. H. GRADWOHL, M.D., Director of the Gradwohl Laboratories and Gradwohl School of Laboratory Technique; Formerly Director of Laboratories, St. Louis County Hospital, etc. Pp. 1607; 492 text illustrations and 44 color plates. Second edition. St. Louis: The C. V. Mosby Company, 1938. Price, $12.50.

FOLLOWING his book on Laboratory Technique, published in 1933 (see Review, this JOURNAL, **184**, 865, 1932), the author in 1935 brought out the first edition of this larger work. This second edition is still larger—in fact even somewhat cumbersome—with amplified sections and new illustrations. The chapter on hematology, for instance, has been increased by 100 pages, 11 pages of which are given to consideration of the unsettled problem of the origin of blood cells, and 7½ more to the physiology of the hematopoietic organs. This seems unnecessary, even out of place, in a book of this kind. Similar inclusions of questionably wide scope add up to a considerable portion of an unwieldy book, with a corresponding effect on the price. On the other hand, the book certainly contains a wealth of information in readily available form.

E. K.

MEDICINE IN MODERN SOCIETY. By DAVID RIESMAN. Pp. 226. Princeton: Princeton University Press, 1938. Price, $2.50.

THIS timely, important and interesting volume is an amplification of the author's recent Vanuxem lectures at Princeton. Without strain, it covers such superficially diverse subjects as the history of medicine—necessarily in very brief survey—cancer, the neuroses, and other aspects of modern and preventive medicine, and the recent developments of socialized medicine.

Yet all follow in logical sequence, combining to elucidate the basic topic of the modern doctor's relation to the patient and to society. The reader will eventually agree with the author's belief that "the history of medicine is in reality an epitome of the history of civilization and should form a part of every man's culture." Striking are the author's 28 items selected as peaks in medical history; of these discoveries only 8 were made more than a century ago, while 10 or more can properly be ascribed to the 20th century. Small wonder, then, that the old relation of doctor to patient has been disturbed and the best new relations not yet firmly established. It is indeed significant when such a wise physician, possessing an extensive knowledge of the history of his profession and of culture in general, strengthened by a long and active career in practical and in organized medicine, concludes that today's medical service to the public is capable of great improvement and that the organized profession has been backward in the movement to remedy the situation. The charm of the author's simple, homely, yet arrestingly illustrative style and the store of knowledge and wisdom exhibited can only be appreciated by reading the book itself. This procedure is highly recommended. E. K.

NEW BOOKS.

Clinical Gastroenterology. By HORACE WENDELL SOPER, M.D., F.A.C.P., St. Louis, Mo. Pp. 314; 212 illustrations. St. Louis: The C. V. Mosby Company, 1939. Price, $6.00.

Memorialia Herman Boerhaave 1738–1938. Optimi Medici. Pp. 133; 16 illustrations. Haarlem: De Erven F. Bohn N.V., 1939. Price, Hfl. 1.90.

The Vaginal Diaphragm. Its Fitting and Use in Contraceptive Technique. By LE MON CLARK, M.S., M.D., Chicago, Illinois. Pp. 107; 53 illustrations. St. Louis: The C. V. Mosby Company, 1939. Price, $2.00.

Whence? Whither? Why? A New Philosophy Based on the Physical Sciences. By AUGUSTA GASKELL. Introduction by F. K. RICHTMYER, Professor of Physics, Cornell University; Dean of the Graduate School. Pp. 312. New York: G. P. Putnam's Sons, 1939. Price, $2.50.

This book aims to answer such questions as: "Is there life after death? Is there a personal God who cares about man? Were we put on this earth for a definite purpose? And if so, what is that purpose? Or does Science discredit the belief in God and immortality? 'Crush the brain and that ends all'; is that true? Can we believe the Bible, in the face of the cold facts of Science?"

Synopsis of Pulmonary Tuberculosis. By JACOB SEGAL, M.D., Physician in Charge of Fordham Hospital Tuberculosis Clinic, New York; Associate Visiting Physician, Riverside Hospital, New York, and Bronx Hospital. Foreword by the Late POL N. CORYLLOS, M.D., F.A.C.S., Professor of Clinical Surgery, Cornell Medical College; Director of Thoracic Surgery at Seaview and Metropolitan Hospitals, New York, etc. Pp. 150; illustrated. New York: Oxford University Press, 1939. Price, $2.75.

League of Nations. Bulletin of the Health Organisation, Vol. 7, Nos. 4 (Aug.) and 5 (Oct.), 1938. Pp.: No. 4, 73; No. 5, 217. New York: Columbia University Press, 1938. Price, 65c each.

No. 4 gives information concerning the activities of the Organisation. Among other items, the volume contains two reports of special interest. The first is by the Commission on Physical Education; the other deals with certain technical aspects of nutrition. No. 5 is specially devoted to the question of biological standardisation. It also contains studies on the international standard of vitamin B and the Report on the Third International Conference on the Standardisation of Hormones.

Studies on the Size of the Red Blood Cells Especially in Some Anaemias. By ERIK MOGENSEN. Pp. 216; 43 illustrations. Copenhagen: Ejnar Munksgaard, 1938; London: Oxford University Press, 1938.

The Medical Clinics of North America, Vol. 23, No. 2 (Baltimore Number—March, 1939). Pp. 294; illustrated. Philadelphia: W. B. Saunders Company, 1939.

Ueber die Integrative Natur der Normalen Hornbildung. Teil III. Systematischer Rückblick. By GOSTA EKEHORN, D:R MED., Stockholm. Pp. 292 Helsingfors: Printed by Mercators Tryckeri, 1938.

Studies of Trauma and Carbohydrate Metabolism with Special Reference to the Existence of Traumatic Diabetes. By VIGGO THOMSEN. Pp. 416; illustrated. Copenhagen: Ejnar Munksgaard, 1938. Price, d. Kr. 15.

Chemie und Physiologie des Eiweisses Mit Unterstützung der Stadt Frankfurt a.M. 3. Frankfurter Konferenz für Medizinisch-naturwissenchaftliche Zusammenarbeit am 2. und 3. Juni, 1938. Herausgegeben von DR. R. OTTO, Geh. Med.-Rat. Direktor des Staatl. Instituts für. exper. Therapie und des Forschungsinstituts für Chemotherapie, Honorarprofessor in der med. Fakultät, Frankfurt a.M., DR. K. FELIX, o. Professor für vegetative Physiologie, Frankfurt a.M., and DR. F. LAIBACH, o. Professor für Botanik, Frankfurt a.M. Pp. 203. Dresden: Theodor Steinkopff, 1938. Price, Paper, Rm. 6.75.

Feminine Hygiene in Marriage. By A. F. NIEMOELLER, A.B., M.A., B.S. With a Foreword by WINFIELD SCOTT PUGH, B.S., M.D. Pp. 155; illustrated. New York: Harvest House, 1938. Price, $2.00.

Problems of Ageing. Biological and Medical Aspects. A Publication of The Josiah Macy, Jr., Foundation. Edited by E. V. COWDRY, Washington University, St. Louis. Twenty-six Contributors. Pp. 758; 120 illustrations. Baltimore: The Williams & Wilkins Company, 1939. Price, $10.00.

An Introductory Guide to Biochemistry. By SIDNEY BLISS, PH.D., Professor of Biochemistry, Tulane University School of Medicine. Pp. 103. Philadelphia: W. B. Saunders Company, 1939. Price, $1.25.

Superfluous Hair and Its Removal. By A. F. NIEMOELLER, A.B., M.A., B.S. With a Foreword by M. H. MORTON, M.D. Pp. 155; illustrated. New York: Harvest House, 1938. Price, $2.00.

You Can't Eat That! A Manual and Recipe Book for Those Who Suffer Either Acutely or Mildly (and perhaps unconsciously) from Food Allergy. By HELEN MORGAN. Foreword by Dr. WALTER C. ALVAREZ, of the Mayo Clinic. Pp. 330. New York: Harcourt, Brace & Co., 1939. Price, $2.50.

Malaria in Panama. (The American Journal of Hygiene, Monographic Series, No. 13, January, 1939.) Supported by the De Lamar Fund of The Johns Hopkins University. By JAMES STEVENS SIMMONS, B.S., M.D., PH.D., S.D., Lt. Col., Medical Corps, U. S. Army, etc. With the Collaboration of George R. CALLENDER, M.D., Lt. Col., Medical Corps, U. S. Army, etc., DALFERES P. CURRY, M.D., Major, Medical Reserve Corps, U. S. Army, etc., SEYMOUR C. SCHWARTZ, B.S., M.D., DR. P.H., Lt. Col., Medical Corps, U. S. Army, etc., and RAYMOND RANDALL, D.V.M., Lt. Col., Veterinary Corps, U. S. Army, etc. Pp. 326; 32 figures and 58 tables. Baltimore: The Johns Hopkins Press, 1939. Price, $1.10.

The Principles and Practice of Ophthalmic Surgery. By EDMUND B. SPAETH, M.D., Associate Professor of Ophthalmology in the Graduate School of Medicine at the University of Pennsylvania; Ophthalmologist to the Orthopedic Hospital and Infirmary for Nervous Diseases, Philadelphia; Consultant in Ophthalmology to the Philadelphia Hospital for the Insane (Byberry), etc. Pp. 835; 413 illustrations. Philadelphia: Lea & Febiger, 1939. Price, $10.00.

PROGRESS
OF
MEDICAL SCIENCE

THERAPEUTICS

UNDER THE CHARGE OF
CARY EGGLESTON, M.D.
ASSISTANT PROFESSOR OF CLINICAL MEDICINE, CORNELL UNIVERSITY MEDICAL COLLEGE, NEW YORK CITY
AND
SOMA WEISS, M.D.
ASSOCIATE PROFESSOR OF MEDICINE, HARVARD MEDICAL SCHOOL, BOSTON, MASS.

ADRENAL CORTICAL HORMONE THERAPY.*

NINE years have elapsed since patients with Addison's disease were first treated with potent extracts of adrenal cortex (Rowntree and Greene,[68] and Hartman, Aaron and Culp[33]), prepared independently by Swingle and Pfiffner,[74] and Hartman and Brownell.[29] During the 4 years subsequent to this discovery, difficulties encountered in the preparation of large quantities of potent extract and in the standardization of the hormone limited clinical trial. However, numerous reports regarding studies in experimental adrenal insufficiency and patients with Addison's disease, treated with adrenal cortical extract, appeared during this period. The beneficial effect of sodium salts (Loeb,[50] and Harrop, Weinstein *et al.*,[27] and the detrimental effect of potassium (Truszkowski and Zwemer,[87] and Wilder, Kendall *et al.*[92]) in adrenal insufficiency were not fully appreciated by most investigators at this time. Thus, in many instances, it is difficult to evaluate the benefits ascribed to hormone therapy when data regarding the mineral intake are not given.

Approximately 5 years ago, adequate quantities of adrenal cortical hormone became available commercially. Since that time nearly every known human disease has been treated with adrenal cortical extract. In a great many instances, the effect of treatment in a single patient has been reported; in other studies, the quantity of material administered was obviously inadequate. From a great number of clinical observations and experiments, there emerge a few conditions in which it appears that adrenal cortical hormone therapy is of benefit.

Recently, Steiger and Reichstein[73] announced the preparation of desoxy-corticosterone acetate from stigmasterol, and subsequently Reichstein and von Euw[66] recovered desoxy-corticosterone from an extract of adrenal cortex. It has been shown (Thorn and Eisenberg[81]) that daily intramuscular injections of this compound will maintain adrenal-

* For previous reviews on this subject the reader is referred to Greene,[16] Grollman[19] and Loeb.[51]

ectomized dogs in excellent condition, despite a diet low in sodium and chloride. The availability in the near future of crystalline preparations of adrenal cortical hormone, synthetically prepared, stimulates a review of the possible applications of adrenal cortical hormone therapy at this time.

Methods of Administering Adrenal Cortical Hormone. 1. *Oral Therapy.* Adrenal cortical extract, in aqueous or saline solution, is largely destroyed during its passage through the intestinal tract (Hartman, Thorn, Durant[37]). Two methods have been adopted for preserving the activity of orally administered hormone: 1, adsorption on charcoal (Grollman, Firor and Grollman[21]); and 2, preservation with glycerol (Hartman and Pohle[30]). The requirement of hormone administered orally in adrenalectomized dogs or patients with Addison's disease, is at least 3 times that required by subcutaneous injection (Thorn, Emerson, Eisenberg[82]).

2. *Subcutaneous, Intramuscular and Intravenous Therapy.* Commercial extracts of adrenal cortex are prepared in a solution of normal saline or dilute alcohol for subcutaneous injection. The preparations are relatively pure, containing 0.5 to 3 mg. of solids per cubic centimeter. For immediate action, these extracts may be injected intravenously. However, the duration of action following intravenous injection is transient (Harrop and Thorn[25]). It is also possible that a resistance to further action of the hormone may arise following intravenous administration (Hartman, Lewis and McConnell [36]).

A crystalline preparation of adrenal cortical hormone, desoxy-corticosterone, has been prepared in a solution of oil for intramuscular injection (Miescher, Fischer and Tschopp[59]). Under local anesthesia, pellets, composed of crystals of desoxy-corticosterone acetate, may be implanted subcutaneously (Thorn, Howard *et al.*[84]).

Standardization of Hormone. Maintenance of adrenalectomized animals appears to be the only reliable test for determining the presence of adrenal cortical hormone in a given preparation. Until crystalline preparations have been proved to be completely supplementary, an absolute standard of biological activity is not practical. For purposes of comparative assay, numerous methods of standardization have been proposed. In general, these are concerned with (*a*) maintenance of normal growth curve of young rats (Hartman and Thorn,[32] Grollman and Firor [20a]), (*b*) maintenance of adrenalectomized dogs (Harrop, Pfiffner, *et al.*[26]) and cats (Hartman and Pohle[30]). In both of these methods the determination of changes in the non-protein nitrogen content of the blood is important; (*c*) tests of muscular efficiency in adrenalectomized animals (Everse, de Fremery,[12] and Ingle[43]) and (*d*) tests based upon the increased susceptibility of adrenalectomized animals to drugs, toxins and physical agents (Perla and Gottesman,[62] and Selye and Schenker[71]). Conditions in which the use of adrenal cortical hormone therapy appears to be beneficial follow:

Addison's Disease. Typical signs and symptoms of Addison's disease rarely are manifest until extensive destruction of functioning adrenal cortical tissue has occurred (Guttman[23]). Thus patients with severe Addison's disease may require almost complete replacement therapy. In all patients with Addison's disease the response to cortical hormone therapy may be modified by several factors (*a*) the type of pathologic

lesion responsible for the adrenal cortical deficiency (Snell[72]), (*b*) the presence of extensive medullary destruction (Harrop[24]) and (*c*) the possibility of permanent changes occurring in other organs as the result of prolonged adrenal insufficiency (Grollman and Firor[20b]).

Early reports (Rowntree, Greene *et al.*,[69] Hartman, Bowen *et al.*,[35] Baird and Albright,[2] and Harrop, Weinstein *et al.*[27]) regarding the use of adrenal cortical extracts in the treatment of Addison's disease were encouraging, and in selected cases very striking. However, in a review of 46 patients treated with adrenal cortical hormone at the Mayo Clinic between 1930 and 1933, Snell[72] noted that the expected length of life was only slightly prolonged although marked and continued improvement was observed in some patients. It is apparent that the failure to improve the average life expectancy of these patients was due in large measure to inadequate treatment resulting from the limited supply of cortical hormone. The low potency of some of the extracts, their prohibitive cost and, in addition, the little recognized need for frequent subcutaneous injections of hormone throughout the day, account for many of the disappointing results with hormone therapy in the treatment of Addison's disease.

During this same period, the strikingly beneficial effect of sodium salts in the treatment of Addison's disease was described by Loeb,[50] and Harrop *et al.*,[27] and as a result, some doubt was expressed as to the value, if any, of adrenal cortical extract therapy in the treatment of Addison's disease. It was evident that patients could be kept alive, and in some cases could be maintained in fairly good condition by means of treatment with large quantities of sodium salts alone. It was also evident that the daily requirement of hormone could be greatly reduced by the addition of sodium salts to the diet of both patients with Addison's disease and animals with experimental adrenal insufficiency. In many patients, however, treatment with added sodium salts and a diet of low potassium content (Wilder *et al.*[92]) was not sufficient for maintenance and, in addition, daily injections of hormone were required (Greene[16]). It is obviously difficult to evaluate the benefits of hormone therapy in patients maintained on a regimen of high sodium and chloride intake combined with small doses of extract.

In 1936 it was shown that adequate quantities of adrenal cortical extract (12 to 30 cat units daily, injected subcutaneously in divided doses), *without supplementary sodium chloride therapy* or reduction in the potassium content of the diet, resulted in a positive sodium and chloride balance, a negative potassium balance, an increase in plasma volume and total plasma content of sodium and chloride, an increase in blood pressure, weight gain, improved appetite and muscular strength (Thorn, Garbutt *et al.*[85b]). Withdrawal of extract treatment promptly resulted in a diuresis associated with an increased renal loss of sodium and chloride decreased renal loss of potassium, reduction in plasma volume and total plasma content of sodium and chloride, reduction in blood pressure, loss of weight and decreased appetite and strength. Restoration of treatment with adrenal cortical extract, alone, caused the disappearance of these signs and symptoms and resulted in a return toward normal. Furthermore, repeated intravenous injections of adrenal cortical extract were found to have an immediate effect on the renal excretion of sodium and potassium in patients with Addison's disease

(Thorn, Garbutt *et al.*[85c]). Thompson, Thompson *et al.*[79] were able to maintain 4 patients with Addison's disease, in good condition, for long periods of time, by means of daily injections of 10 to 20 cc. of a potent commercial adrenal cortical extract (this quantity of extract being derived for 750 to 1500 gm. of beef adrenal). No added sodium chloride therapy or reduction in diet content of potassium was used. It appears from these studies that adequate hormone therapy can result in restoration of patients with Addison's disease to normal activity without regard to the mineral intake. However, the cost of this type of treatment and the necessity for frequent subcutaneous injections limit both its applicability and desirability.

Oral administration of adrenal cortical extract is efficacious in the treatment of animals with experimental adrenal insufficiency (Britton, Flippin, and Silvette,[5] Grollman and Firor,[20a] Hartman and Pohle[30]), and in the treatment of patients with Addison's disease (Thorn, Emerson and Eisenberg[82]). For patients with Addison's disease the requirement of orally administered hormone, in either glycerol solution or as a charcoal adsorbate, is at least 3 times the quantity of hormone necessary when injected subcutaneously in aqueous solution. Effective oral treatment for most patients with severe Addison's disease is impractical at present because of the cost.

Crystalline compounds, corticosterone and dehydro-corticosterone isolated by Mason, Meyers and Kendall,[56] and de Fremery, Laquer *et al.*[14] from extracts of the adrenal cortex, have been reported to maintain adrenalectomized animals in good condition. These compounds have not been available for clinical trial. The desirability of employing crystalline preparations of known chemical composition in the treatment of Addison's disease is evident. In 1937, Steiger and Reichstein[73] announced the preparation of desoxy-corticosterone acetate from stigmasterol, and recently Reichstein and von Euw[66] succeeded in isolating desoxy-corticosterone from an extract of adrenal cortex, thus establishing the natural occurrence of this compound. The synthetically prepared compound was shown to be capable, on injection, of maintaining adrenalectomized dogs in good condition, despite a diet low in sodium and chloride (Thorn, Engel and Eisenberg,[83a] Thorn and Eisenberg[81]). Studies on 8 patients with Addison's disease treated with daily intramuscular injections of desoxy-corticosterone acetate in sesame oil (Thorn, Howard and Emerson[86]), indicate that treatment with this compound (5 to 25 mg. daily) resulted, in all of the patients, in sodium and chloride retention, increased potassium excretion, increased plasma volume and total plasma content of sodium and chloride, an increase in body weight and blood pressure, improved appetite and strength. These changes occurred without supplementary treatment with sodium salts or decreased potassium content of the diet. Withdrawal of desoxy-corticosterone acetate treatment resulted in a diuresis, loss of sodium and chloride, decreased excretion of potassium, decrease in plasma volume with hemoconcentration and a decrease in total plasma content of sodium and chloride, loss in weight and decrease in appetite and strength. Institution of treatment with desoxy-corticosterone acetate, following a period of withdrawal resulted again in all of the changes previously observed. As far as could be determined, the treatment with desoxy-corticosterone acetate resembled in every way the effect of treatment

with potent adrenal cortical extract. It appeared from these studies that 1 mg. of desoxy-corticosterone acetate, in oil, injected once daily intramuscularly, was equivalent to approximately 3 cc. of a potent commercial adrenal cortical extract injected subcutaneously, the latter being administered in divided doses. Levy-Simpson,[48] noted that desoxy-corticosterone acetate treatment was qualitatively similar to that of adrenal cortical extract treatment in 2 patients with Addison's disease.

Applying the technique which Deanesly and Parkes[9] employed in experimental studies on sex hormone administration, Thorn, Engel and Eisenberg[83b] recently demonstrated that the subcutaneous implantation of pellets of crystalline desoxy-corticosterone acetate is a practical and very economical method of administering this hormone to adrenalectomized dogs. Following the implantation of pellets of desoxy-corticosterone acetate, the animals were maintained in excellent condition for long periods, despite a diet low in sodium and chloride. Preliminary experiments in which this technique was applied clinically indicate results equally as successful as those observed in experimental animals (Thorn, Howard *et al.*[84]). Such a form of treatment provides for a continuous supply of hormone and the studies demonstrated a considerable saving of hormone. If this form of therapy proves to be practical, patients with Addison's disease could be treated effectively by this method at moderate cost.

Although striking changes in the clinical condition of patients have followed continued treatment with either potent adrenal cortical extract or synthetically prepared desoxy-corticosterone acetate, changes in the pigmentation of patients with Addison's disease has been neither uniform nor predictable (Greene,[16] Hartman *et al.*,[38] Thorn *et al.*[86]). It is possible that intensive hormone therapy has not been continued long enough, since, for practical purposes, most patients are maintained on large doses of sodium salts and relatively small quantities of hormone. It is also possible that the preparations which have been used may not contain the factor which influences pigmentation, even though these preparations do possess life-maintaining property. It has also been suggested that changes in pigmentation may be related to disturbances in function of the medulla (Harrop[24]). However, pathologic data provide conflicting evidence on this point (Greenhow,[18] Barker[3]). Szent-Gyorgyi[76] has suggested the use of ascorbic acid in conjunction with adrenal cortical extract therapy in the treatment of pigmentation in patients with Addison's disease. Szule[77] has reported favorably on this method of treatment. In the author's experience (unpublished data) ascorbic acid administered by mouth or parenterally has been ineffective in significantly altering the pigmentation of patients with Addison's disease.

It has been shown by Kendall *et al.*[46] that disturbances in the concentration and distribution of sodium and potassium in the body result in altered carbohydrate metabolism. However, even at a time when electrolyte concentration and distribution is apparently normal, patients may, on occasion, develop hypoglycemia. It cannot be stated at this time whether a persistence in the disturbance of carbohydrate metabolism indicates (*a*) lack of intensive hormone therapy (*b*) an absence of a necessary factor in the preparations now employed or (*c*) a manifestation

of a disturbance in adrenal medullary function. The observations of Lukens and Dohan,[53] and of Long, Fry and Thompson[52] render the second of these three possibilities unlikely. For practical purposes, it seems advisable at present, to maintain all patients with Addison's disease on a diet high in readily available carbohydrate.

The success of treatment in Addison's disease depends in part upon the etiologic factor responsible for the changes in the adrenals, and to a large measure upon the patient's ability to follow prescribed treatment. Successful treatment should permit the patient to resume his normal occupation. Maintenance of life alone should not be considered sufficient. In some patients, the only treatment necessary is added sodium chloride or a combination of sodium salts; others may need restriction of potassium intake in addition to added sodium chloride therapy; most patients, in order to resume normal activity, will require hormone therapy. Patients receiving hormone therapy, rarely need to suffer the inconvenience imposed by adhering to a diet, low in potassium; added sodium chloride therapy will greatly reduce the hormone requirement. It has not been demonstrated conclusively that full replacement with hormone therapy offers advantages over combined hormone and mineral therapy. The high cost of hormone preparations will prevent most patients from being treated with hormone alone. Hormone may be given orally, but in severe Addison's disease, since this method requires at least three times the quantity of extract necessary by subcutaneous injection, it is usually too costly. Administration of extract in divided doses is much more efficacious than a single daily injection. Intravenous injections of extract, exert only a transient effect and may result after repeated injections, in the formation of substances antagonistic to the hormone action (Hartman *et al.*[36]) These changes do not occur following the subcutaneous injection of adrenal cortical extract (Harrop and Thorn[25]). For this reason, intravenous administration of extract should be reserved for emergency treatment.

Surgical Operations on Patients with Addison's Disease. By using large doses of adrenal cortical extract, 40 cc. daily, in divided doses for 2 days preoperatively, 20 cc. of extract immediately after operation, and 10 cc. twice daily subsequently, in addition to intravenous sodium chloride, Greene, Walters and Rowntree[17] were able to perform successfully an epididymectomy and orchidectomy for tuberculosis in a patient with Addison's disease. The great improvement which follows intensive therapy in patients with Addison's disease, justifies the consideration of the surgical removal of a focus of tuberculosis. This encouraging report indicates the possibility of performing such operations successfully in patients with Addison's disease.

Acute Adrenal Insufficiency. A. *Associated with surgical procedures involving the adrenal gland.* Exploration or denervation of the adrenals, resection of adrenal tissue for hypertension, and removal of adrenal tumors may result in symptoms of acute adrenal insufficiency, either as a result of tissue removed, or as a consequence of hemorrhage into the gland. Intensive therapy (intravenous saline and glucose, and large quantities of adrenal cortical extract 50 to 150 cc. daily, injected intravenously and subcutaneously) may be required in order to effect recovery (Walters and Kepler,[88] and Prather[64]).

B. *Bilateral adrenal hemorrhage* with death in 24 to 48 hours has been reported in 1% of autopsies on new-born infants (Levinson[47]). In a series of 8 cases, 6 were noted to have had breech delivery and 2 followed Cesarean section. In the latter, rather vigorous slapping over the lumbar region had been used in attempting resuscitation. As yet, no instance of treatment in this disorder has been reported. If the diagnosis were suspected, immediate institution of intensive treatment would seem to be indicated.

C. *Waterhouse–Friderichsen Syndrome.* The occurrence of extensive hemorrhage in the adrenals during the course of meningococcemia, scarlet fever, diphtheria, pneumonia and poliomyelitis is well known (Aegerter[1]). In many patients with this complication the infection may be adequately controlled by chemical or serum therapy and the life of the patient then depends upon the successful treatment of acute adrenal insufficiency. Bilateral adrenal hemorrhage, frequently complicates the fatal termination of acute pemphigus. Recently, Talbott[78] has treated 5 patients suffering from acute pemphigus by means of 10 to 15 cc. daily of adrenal cortical extract, administered subcutaneously in divided doses, and 1500 cc. of normal saline intravenously daily. A satisfactory remission occurred in each case. Normal saline solution, alone, was not sufficient. Five control cases died within 5 weeks after admission to the hospital. Two of these patients were examined postmortem and found to have bilateral adrenal hemorrhages. Obviously, adrenal insufficiency is not the etiologic factor in acute pemphigus, but this report indicates the possibility of treating the acute bilateral adrenal hemorrhage which not infrequently complicates this condition. Hormone treatment of the Waterhouse-Friderichsen syndrome in infections which are readily amenable to serum or chemotherapy would seem to provide a promising possibility of therapeutic response.

Diagnosis of Adrenal Insufficiency. The desirability of undertaking treatment early in the course of adrenal insufficiency is readily understood. However, the difficulty of establishing a diagnosis of adrenal insufficiency in patients who do not present all of the classical signs and symptoms of Addison's disease is well known. Three types of procedures have been suggested as an aid in the diagnosis of adrenal insufficiency: 1, the precipitation of the signs and symptoms of adrenal insufficiency following a period of sodium chloride withdrawal (Harrop *et al.*[27]); 2, the estimation of the concentration of sodium and chloride in the urine, under standard conditions (Cutler, Power and Wilder[8]); and 3 the effect of adrenal cortical hormone on the renal excretion of electrolytes (Thorn, Garbutt *et al.*[85b]). It is apparent that no one of these tests is infallible. The first procedure is relatively simple, but may end disastrously (Lillienthal[49]). The second involves a relatively simple metabolic study and necessitates only the determination of urinary chloride. Again, however, this test may result in the precipitation of signs of acute adrenal insufficiency. The third procedure is safe, but involves careful balance studies conducted in a metabolism unit and hence is not generally applicable.

Gordon, Sevringhaus and Stark[15] have studied the effect of adrenal cortical hormone therapy on the blood pressure and symptomatic response of 32 patients who complained of asthenia, as the presenting symptom. Seventeen of these patients were definitely benefited by

hormone administration, and subsequently have been maintained on sodium salts of adrenal cortical extract with marked benefit. It was thought by these authors that the symptoms and hypotension of the group responding to hormone therapy indicated adrenal insufficiency.

Post Infectious Asthenia. Changes in the adrenals (focal necroses and hemorrhages, cloudy swelling and congestion) are known to occur during the course of acute infections (MacKenzie,[54] Cowie and Beaven[6]). It is possible that in some patients, the marked prostration and hypotension which persist after the subsidence of infection may, in part, be due to adrenal insufficiency. Robbins[67] has reported the successful treatment of post-infectious asthenia following acute streptococcic infections. Hartman, Beck and Thorn[34] have reported improvement (as measured by the ergometer) in post-infectious asthenia. The latter authors point out the difficulty of obtaining suitable control periods. It appears probable that adrenal cortical hormone therapy, or sodium salts, or a combination of both forms of treatment may be efficacious in alleviating post-infectious asthenia in selected cases. However, the difficulty in obtaining critical data regarding the efficacy of any form of therapy in this syndrome is apparent.

Circulatory Shock. The presence of anhydremia and shock in the crisis of adrenal insufficiency has suggested the possibility of using adrenal cortical hormone in the prevention or treatment of shock initiated by other causes. Swingle, Parkins *et al.*[75] have demonstrated the value of adrenal cortical hormone therapy in protecting adrenalectomized dogs from the shock which follows intestinal manipulation. Heuer and Andrus[39] have shown that 10 to 40 cc. of a commercial adrenal cortical extract protected normal dogs from the shock induced by injecting aqueous extracts of closed intestinal loops. Treated animals lost 8.5% of their plasma volume whereas untreated animals lost 30.5%. Epinephrin, cholesterin or vitamin C were ineffective. Treatment with adrenal cortical extract was given either prior to, or simultaneously with the injection of the noxious agent. Attempts to restore shocked animals, by means of adrenal cortical extract were ineffective. Restoration by means of transfusion or a combination of cortical hormone and transfusion was successful. Greene[16] notes the favorable effect of adrenal cortical hormone therapy in a patient in shock following partial intestinal obstruction with vomiting and dehydration. MacLean, Sullivan and Zwemer[55] have reported a satisfactory therapeutic response following the use of adrenal cortical extract in the treatment of children with acute gastro-enteritis. In a series of 50 patients treated with injections of adrenal cortical extract (1 to 2 cc. every 4 to 12 hours) preoperatively and postoperatively, Reed has reported maintenance of blood electrolytes, stabilization of blood pressure and rapid healing of incisions. It seems unlikely that such small quantities of extract could have any appreciable effect.

Etherization of normal dogs results in a reduction in plasma volume of approximately 12% (McAllister[57]). This fluid shift may be prevented by repeated intravenous injections of adrenal cortical extract prior to and during the administration of ether (McAllister and Thorn[58]). Dragstedt, Mills and Mead[10] noted that adrenal cortical extract treatment in experimental animals greatly ameliorated the symptoms of

anaphylactic shock without reducing the incidence. Again in these experiments, the adrenal cortical extract was given prior to the shocking dose of serum.

Severe burns in experimental animals have been treated by means of adrenal cortical extract (Einhauser[11]). Although this author reports an increase in the survival period of animals treated with adrenal cortical extract ($\frac{1}{3}$ cc. of extract, per animal, per day), it seems unlikely that this small quantity of material would have any significant effect. Wilson, Rowley and Gray[93] using 1 to 2 cc., hourly, of commercial adrenal cortical extract report the beneficial effect of this treatment in 3 infants with intensive burns. They state that suprarenal cortical hormone should be used only as adjuvant treatment. Hartman[28] has reported the beneficial effect of large doses of adrenal cortical extract in the treatment of shock occurring in an infant following severe burns. It is difficult to determine the benefit derived from adrenal cortical extract treatment in patients with severe burns because of the inability to study suitable control cases and because of the desirability of including the supportive measures generally indicated. Animal experiments would suggest that adrenal cortical hormone therapy might be expected to reduce the incidence or intensity of shock if such therapy were administered prior to the shocking procedure. The beneficial effect of adrenal cortical hormone therapy in the prevention of shock in experimental animals is due, in part at least, to the preservation of plasma volume. There is little evidence, except in *adrenalectomized animals*, to show that adrenal cortical hormone therapy is of any benefit when given after the symptoms of shock have appeared.

Acute Infections. Adrenal cortical hormone therapy increases the resistance of *adrenalectomized animals* to infection (Hartman and Scott[31]). This is considered to be largely related to the general inprovement in the condition of the animals. However, Wenner and Cone[91] have reported that the opsonic power of serum of both suprarenalectomized and normal animals is increased by injections of cortical extract. Furthermore, Wenner[90] reported marked success in the treatment of experimental maxillary sinusitis with adrenal cortical extracts. Scott, Bradford *et al.*[70] have shown that adrenal cortical extract treatment was of *no benefit* in (*a*) protecting normal guinea pigs from the effect of diphtheria toxin (*b*) protecting normal rats from trypanosome infection or (*c*) protecting normal mice from pneumococcus infection. Although the quantity of extract used in these studies was small ($\frac{1}{2}$ the maintenance dose for adrenalectomized guinea pigs being injected daily in the infected animals), this dose of hormone corresponds closely to that which has been used in clinical studies (Najib-Farah[61]). Thus far, with the doses employed, no data have been presented which conclusively demonstrate the effectiveness of adrenal cortical hormone in the treatment of acute infections in the human being.

Disturbances in Muscle Metabolism and Thyroid Function. Adrenal cortical extract therapy has been tried in muscular atrophy, muscular dystrophy and myasthenia gravis (Hartman, Beck and Thorn,[34] Greene,[16] Bernhardt and Levy-Simpson[4]). Apart from a slight temporary improvement in some patients, the course of the disease has been unaffected by this form of therapy. Weinstein and Marlow[89] failed to observe any

beneficial effect of adrenal cortical extract, injected daily, in 17 patients with hyperthyroidism and in 2 patients with hypothyroidism. The dosage used was 9.5 cc. per day per patient (average) which represented 285 gm. of suprarenal cortex. Furthermore, no beneficial effect was observed in 3 patients with hyperthyroidism who were fed 18.6 gm. per day of raw suprarenal cortex.

Miscellaneous Conditions. Hoskins and Freeman[42a] report that 1 gm. three times daily, of a glycerinated adrenal cortical extract, resulted after 3 weeks of treatment in an average weight gain of 3 kg. in 17 schizophrenic patients. The weight of 17 control normal subjects was unaffected by this treatment. Studies of this extract indicate that "cortin" is probably not the factor in the extract responsible for the change (Hoskins and Gottlieb[42b]).

As stated earlier in this review, adrenal cortical extract therapy has been tried in a wide variety of clinical conditions, *i. e.*, vomiting of pregnancy (Kemp[45a]), asthma (Fineman,[13] Pottenger[63]), psoriasis (Gruneberg[22]) and glaucoma (Josephson[44]). Varying degrees of success are reported in these conditions following adrenal cortical extract therapy. However, the data presented usually do not permit critical evaluation of the benefits ascribed.

Effect in Normal Human Subjects. Repeated intravenous injections of adrenal cortical extract (20 cat units hourly, for 4 hours) in normal human subjects resulted in an immediate effect on the renal excretion of electrolytes (Thorn, Garbutt *et al.*[85a]). A marked decrease in the renal excretion of sodium and chloride, and an increased excretion of potassium were noted. Subsequently, it was shown that repeated intravenous injections of adrenal cortical hormone, throughout the day, resulted in a similar change in the 24-hour renal excretion of electrolytes (Thorn[80]). Similar effects have been observed in normal dogs following the intravenous or subcutaneous injection of large quantities of adrenal cortical extract (Harrop and Thorn[25]) and following the subcutaneous injection of 1 to 5 mg. of crystalline desoxy-corticosterone acetate in oil (Thorn, Engel and Eisenberg[83]).

Hitchcock and Thorn[40] noted that injections of adrenal cortical extract in normal subjects resulted in a pronounced drop in oxygen consumed in maintaining the erect posture. Hitchcock, Grubbs and Hartman[41] have confirmed this observation, and, in addition, have noted that a marked depression occurred in the oxygen consumption of subjects walking on a treadmill at the rate of 100 meters per minute. Missuro, Dill and Edwards[60] noted that the efficiency with which easy walking was performed was increased for some days after injections of adrenal cortical extract.

Summary and Conclusions. Adrenal cortical hormone is of definite value in the treatment of Addison's disease and conditions associated with acute adrenal insufficiency. The response to adrenal cortical therapy may be an aid in the diagnosis of adrenal insufficiency. It is possible that adrenal cortical hormone therapy may be of benefit in other disorders, but, to date, conclusive evidence has not been presented to substantiate this suggestion. Injections of adrenal cortical hormone have been shown to have a definite effect in normal human subjects.

GEORGE W. THORN, M.D.

REFERENCES.

(1.) **Aegerter, E. E.**: J. Am. Med. Assn., **106**, 1715, 1936. (2.) **Baird, P. C., Jr.**, and **Albright, F.**: Arch. Int. Med., **50**, 394, 1932. (3.) **Barker, N. W.**: Arch. Path., **8**, 432, 1929. (4.) **Bernhardt, H.**, and **Levy-Simpson, S.**: Klin. Wchnschr., **11**, 2069, 1932. (5.) **Britton, S. W., Flippin, J. C.**, and **Silvette, H.**: Am. J. Physiol., **99**, 44, 1931. (6.) **Cowie, D. M.**, and **Beaven, P. W.**: Arch. Int. Med., **24**, 78, 1919. (7.) **Crooke, A. C.**, and **Russell, D. S.**: J. Path. and Bact., **40**, 255, 1935. (8.) **Cutler, H. H., Power, M. H.**, and **Wilder, R. M.**: J. Am. Med. Assn., **111**, 117, 1938. (9.) **Deanesly, R.**, and **Parkes, A. S.**: Proc. Roy. Soc. London, s. B, **124**, 279, 1937. (10.) **Dragstedt, C. A., Mills, M. A.**, and **Mead, F. B.**: J. Pharm. and Exp. Ther., **59**, 359, 1937. (11.) **Einhauser, M.**: Klin. Wchnschr., **17**, 127, 1938. (12.) **Everse, J. W. R.**, and **de Fremery, P.**: Acta brevia neevl., **2**, 152, 1932. (13.) **Fineman, A. H.**: J. Allergy, **4**, 182, 1933. (14.) **de Fremery, P., Laqueur, E., Reichstein, T., Spanhoff, R. W.**, and **Wyldert, I. E.**: Nature, **139**, 26, 1937. (15.) **Gordon, E. S., Sevringhaus, E. L.**, and **Stark, M. E.**: Endocrinology, **22**, 45, 1938. (16.) **Greene, C. H.**: Arch. Int. Med., **59**, 759, 1937. (17.) **Greene, C. H., Walters, W.**, and **Rowntree, L. G.**: Ann. Surg., **98**, 1013, 1933. (18.) **Greenhow, E. H.**: Trans. Path. Soc., London, **24**, 238, 1873. (19.) **Grollman, A.**: The Adrenals, Baltimore, The Williams & Wilkins Company, 1935. (20.) **Grollman, A.**, and **Firor, W. M.**: (*a*) J. Biol. Chem., **100**, 429, 1933; (*b*) Am. J. Physiol., **112**, 310, 1935. (21.) **Grollman, A., Firor, W. M.**, and **Grollman, E.**: J. Biol. Chem., **109**, 189, 1935. (22.) **Gruneberg, T.**: Klin. Wchnschr., **12**, 1908, 1933. (23.) **Guttman, P. H.**: Arch. Path., **10**, 742, 895, 1930. (24.) **Harrop, G. A.**: Ann. Int. Med., **6**, 1579, 1933. (25.) **Harrop, G. A.**, and **Thorn, G. W.**: J. Exp. Med., **65**, 757, 1937. (26.) **Harrop, G. A., Pfiffner, J. J., Weinstein, A.**, and **Swingle, W. W.**: Proc. Soc. Exp. Biol. and Med., **29**, 449, 1932. (27.) **Harrop, G. A., Weinstein, A., Soffer, L. J.**, and **Trescher, J. H.**: J. Am. Med. Assn., **100**, 1850, 1933. (28.) **Hartman, F. A.**: Ann. Int. Med., **7**, 6, 1933. (29.) **Hartman, F. A.**, and **Brownell, K. A.**: Proc. Soc. Exp. Biol. and Med., **27**, 938, 1930. (30.) **Hartman, F. A.**, and **Pohle, W. D.**: Endocrinology, **20**, 795, 1936. (31.) **Hartman, F. A.**, and **Scott, W. J. M.**: J. Exp. Med., **55**, 63, 1932. (32.) **Hartman, F. A.**, and **Thorn, G. W.**: Proc. Soc. Exp. Biol. and Med., **28**, 94, 1930. (33.) **Hartman, F. A., Aaron, A. H.**, and **Culp, J. E.**: Endocrinology, **14**, 438, 1930. (34.) **Hartman, F. A., Beck, G. M.**, and **Thorn, G. W.**: J. Nerv. and Ment. Dis., **77**, 1, 1933. (35.) **Hartman, F. A., Bowen, B. D., Thorn, G. W.**, and **Greene, C. W.**: Ann. Int. Med., **5**, 539, 1931. (36.) **Hartman, F. A., Lewis, L. A.**, and **McConnell, K. P.**: Endocrinology, **24**, 197, 1939. (37.) **Hartman, F. A., Thorn, G. W.**, and **Durant, R. R.**: Ibid., **21**, 516, 1937. (38.) **Hartman, F. A., Thorn, G. W., Lockie, L. M., Greene, C. W.**, and **Bowen, B. D.**: J. Am. Med. Assn., **98**, 788, 1932. (39.) **Heuer, G. F.**, and **Andrus, W. De W.**: Ann. Surg., **100**, 734, 1934. (40.) **Hitchock, F. A.**, and **Thorn, G. W.**: J. Nutr., **11**, 15, suppl. abstr., 1936. (41.) **Hitchcock, F. A., Grubbs, R. C.**, and **Hartman, F. A.**: Am. J. Physiol., **121**, 542, 1938. (42*a*.) **Hoskins, R. G.**, and **Freeman, H.**: Endocrinology, **20**, 565, 1936. (42*b*.) **Hoskins, R. G.**, and **Gottlieb, J. S.**: Ibid., p. 188. (43.) **Ingle, D. J.**: Am. J. Physiol., **116**, 622, 1936. (44.) **Josephson, E. M.**: Eye, Ear, Nose and Throat Monthly, **13**, 453, 1935. (45.) **Kemp, W. N.**: (*a*) Endocrinology, **16**, 434, 1932; (*b*) Canad. Med. Assn. J., **28**, 389, 1933. (46.) **Kendall, E. C., Flock, E. V., Bollman, J. L.**, and **Mann, F. C.**: J. Biol. Chem., **126**, 697, 1938.

(47.) **Levinson, S. A.**: Am. J. Surg., **29**, 94, 1935. (48.) **Levy-Simpson, S.**: Lancet, **2**, 557, 1938. (49.) **Lillienthal, A.**: J. Am. Med. Assn., **110**, 804, 1938. (50.) **Loeb, R. F.**: Proc. Soc. Exp. Biol. and Med., **30**, 808, 1933. (51.) **Loeb, R. F.**: J. Am. Med. Assn., **104**, 299, 1935. (52.) **Long, C. N. H., Fry, E. G.**, and **Thompson, K. W.**: Proc. Am. Physiol. Soc., Am. J. Physiol., **123**, 130, 1938. (53.) **Lukens, F. D. W.**, and **Dohan, F. C.**: Endocrinology, **22**, 51, 1938. (54.) **MacKenzie, J. J.**: Endocrin. and Metab., **2**, 257, 1922. (55.) **MacLean, A. B., Sullivan, R. C.**, and **Zwemer, R. L.**: Am. J. Dis. Child., **43**, 1277, 1932. (56.) **Mason, H. L., Meyers, C. S.**, and **Kendall, E. C.**: J. Biol. Chem., **116**, 267, 1936. (57.) **McAllister, F. F.**: Am. J. Physiol., **124**, 391, 1938. (58.) **McAllister, F. F.**, and **Thorn, G. W.**: Proc. Soc. Exp. Biol. and Med., **36**, 736, 1937. (59.) **Miescher, K., Fischer, W. H.**, and **Tschopp, E.**: Nature, **142**, 435, 1938. (60.) **Missuro, V., Dill, D. B.**, and **Edwards, H. T.**: Am. J. Physiol., **121**, 549, 1938. (61.) **Najib-Farah**: Lancet, **1**, 777, 1938. (62.) **Perla, D.**, and **Gottesman, J. M.**: Proc. Soc. Exp. Biol. and Med., **28**, 650, 1931. (63.) **Pottenger, F. M., Jr., Pottenger, R. T.**, and **Pottenger, F. N.**: California and West. Med., **43**, 10, 1935. (64.) **Prather, G. C.**: New England J. Med., **208**, 872,

1933. (65.) **Reed, F. R.**: Am. J. Surg., **40**, 514, 1938. (66.) **Reichstein, T.**, and **von Euw, J.**: Helv. Chim. Acta, **21**, 1197, 1938. (67.) **Robbins, J. H.**: J. Am. Med. Assn., **100**, 657, 1933. (68.) **Rowntree, L. G.**, and **Greene, C. H.**: Science, **72**, 482, 1930. (69.) **Rowntree, L. G., Greene, C. H., Ball, R. G., Swingle, W. W.**, and **Pfiffner, J. J.**: J. Am. Med. Assn., **97**, 1446, 1931. (70.) **Scott, W. J. M., Bradford, W. L., Hartman, F. A.**, and **McCoy, O. R.**: Endocrinology, **17**, 529, 1933. (71.) **Selye, H.**, and **Schencker, V.**: Proc. Soc. Exp. Biol. and Med., **39**, 518, 1938. (72.) **Snell, A. M.**: Internat. Clin., **3**, 46, 1934. (73.) **Steiger, M.**, and **Reichstein, T.**: Helv. Chim. Acta, **20**, 1164, 1937. (74.) **Swingle, W. W.**, and **Pfiffner, J. J.**: Science, **71**, 321, 1930. (75.) **Swingle, W. W., Parkins, W. M., Taylor, A. R.**, and **Hays, H. W.**: Proc. Soc. Exp. Biol. and Med., **37**, 601, 1937. (76.) **Szent-Gyorgyi, A. V.**: Am. J. Physiol., **90**, 536, 1929. (77.) **Szule, D.**: Deutsch. med. Wchnschr., **59**, 651, 1933. (78.) **Talbott, J. H.**: Personal communication. (79.) **Thompson, W. O., Thompson, P. K., Taylor, S. G.**, and **Hoffman, W. S.**: Proc. Inst. of Med., Chicago, **11**, 202, 1937. (80.) **Thorn, G. W.**: Proc. Soc. Exp. Biol. and Med., **36**, 361, 1937. (81.) **Thorn, G. W.**, and **Eisenberg, H.**: Endocrinology. In Press. (82.) **Thorn, G. W., Emerson, K., Jr.**, and **Eisenberg, H.**: Ibid., **23**, 403, 1938. (83.) **Thorn, G. W., Engel, L. L.**, and **Eisenberg, H.**: (*a*) J. Exp. Med., **68**, 161, 1938; (*b*) Bull. Johns Hopkins Hosp., **64**, 155, 1939. (84.) **Thorn, G. W., Howard, R. P., Emerson, K., Jr.**, and **Firor, W. M.**: Bull. Johns Hopkins Hosp. In Press. (85.) **Thorn, G. W., Garbutt, H. R., Hitchcock, F. A.**, and **Hartman, F. A.**: (*a*) Proc. Soc. Exp. Biol. and Med., **35**, 247, 1936; (*b*) Endocrinology, **21**, 202, 1937; (*c*) Ibid., **21**, 213, 1937. (86.) **Thorn, G. W., Howard, R. P.**, and **Emerson, K., Jr.**, J. Clin. Invest. To be published. (87.) **Truszkowski, R.**, and **Zwemer, R. L.**: Biochem. J., **30**, 1345, 1936. (88.) **Walters, W.**, and **Kepler, E. J.**: J. Am. Med. Assn., **111**, 1061, 1938. (89.) **Weinstein, A.**, and **Marlow, A.**: Bull. Johns Hopkins Hosp., **52**, 408, 1933. (90.) **Wenner, W. F.**: Arch. Otolaryngol., **17**, 774, 1933. (91.) **Wenner, W. F.**, and **Cone, A. J.**: Ibid., **20**, 178, 1934. (92.) **Wilder, R. N., Kendall, E. C., Snell, A. M., Kepler, E. J., Rynearson, E. H.**, and **Adams, M.**: Arch. Int. Med., **59**, 367, 1937. (93.) **Wilson, W. C., Rowley, G. D.**, and **Gray, N. A.**: Lancet, **1**, 1400, 1936.

RADIOLOGY

UNDER THE CHARGE OF
ALBERT MILLER, M.D.,
AND
CHARLES G. SUTHERLAND, M.D.,
CONSULTING PHYSICIANS, SECTION ON ROENTGENOLOGY, THE MAYO CLINIC, ROCHESTER, MINN.

ROENTGENOLOGY IN THORACIC LESIONS.

"DURING the development of any particular branch of medical science there are, of necessity, periods of experimentation in which various angles of the problems encountered are explored. Eventually the time is reached when it becomes advisable to compile all the essential data that have accumulated and to integrate the essential facts into a satisfactory and workable scheme." With this introduction, Pesquera and Sampson[9] suggest that there is, perhaps, nothing of greater importance in the field of thoracic roentgenography at this time than acceptance of a standardized technique that will make available, for the profession at large, comparable thoracic roentgenograms. Tracing the history of roentgenography of the thoracic cage from the discovery of the Roentgen rays, some 40 years ago, these authors present briefly the steps leading up to the present-day concept of a good thoracic roentgenogram. Such a concept, they point out, should represent the result obtained after the

proper manipulation of voltage, milliamperage, time of exposure, focal distances and the focal spot. The individual characteristic and the cause and the effect of each of these factors must be understood.

Their experience has been practical, and whatever conclusions they have drawn are based on the study of roentgenograms. In their studies they paid particular attention to the following points: (1) time of exposure; (2) penetration (contrast); (3) target-to-film distance; and (4) definition of shadows. They found that a satisfactory roentgenogram could be made with a $\frac{1}{10}$ to $\frac{1}{20}$ second exposure. In the roentgenogram, penetration is revealed by the relative value of the blacks and the whites and half-tones. It is by means of the relative values of the shadow that the physician determines the activity of a given pathologic process. The merging of the shadow with the surrounding normal tissue may be gradual or abrupt, and students of the roentgenogram feel reasonably secure in drawing conclusions therefrom, one way or the other. The differentiation of caseous and exudative lesions, in the presence of tuberculosis, from those of the proliferative and fibrous type is a matter of shadow-contrast evaluation. It is conceivable that if improper technique produced greater contrast, the physician might be misled in his deductions. It was a common occurrence, in the experience of Pesquera and Sampson, to have a roentgenogram which exhibited too much contrast suggest a reactivation of a focus, whereas a film of the proper shadow values would not convey this impression. On the basis of differentiation of tissue densities only, it would seem that the use of a grid for thoracic roentgenography should have an advantage; but there are equally important factors which render the use of a grid unsatisfactory. For example, stereoroentgenograms cannot be made with facility; also the occurrence of objectionable grid marks is likely to mar the daily output.

As target-to-film distances increase, undesirable enlargement becomes progressively diminished; according to Wilsey,[11] "Most of the decrease in enlargement has occurred within the four foot distance, beyond this the enlargement diminishes very slowly." On the other hand, as the distance increases, the area of the focal spot also must increase in proportion to the square of the focal film distance which, in itself, tends to nullify any gain in sharpness from long distances unless the efficiency of the focal spot (current-carrying capacity) is increased by greater anode slope, by anode rotation or by both.

Pesquera and Sampson credit Files[4] with concisely summing up the definition by his stating that the best diagnostic results can be obtained only when a tube is used which has the smallest possible focal spot consistent with the part to be roentgenographed. Hence, the physician can confidently rely on small focus tubes (preferably rotary targets), moderate kilovoltages, 5 to 7 foot distances, and $\frac{1}{10}$ to $\frac{1}{30}$ of a second exposure times for the finest of stereoroentgenograms.

The relative cost of equipment necessary to produce relative gains in definition (sharpness of shadows) is presented by the previously mentioned authors in graphic form. They feel that it is now possible for the roentgenologist to obtain a roentgenogram which will enable him to make the diagnosis of early pulmonary tuberculosis and to follow the progress of his patient intelligently during the life of the patient. They believe that a standard diagnostic thoracic roentgenogram can

be made an accomplished fact and that it can efface Sir William Osler's statement: "More than any others, *some* roentgenologists need the salutary lessons of the dead house to correct their visionary interpretations of shadows."[8]

In the Hickey Lecture of 1938, Holmes,[6] speaking of hemoptysis and its roentgenologic diagnosis, said: "No doubt the frequency with which this symptom appears as a presenting symptom varies widely in different clinics and with different physicians." His own experience agreed with that of Dr. Richard Cabot, who once said that from his observations at the Massachusetts General Hospital, the most common cause of blood-spitting was cardiac disease. To cardiac disease, Holmes would add valvular disease, pulmonary emboli and allied conditions. In general practice, he observed, tuberculosis still ranked among the first as a cause. The speaker had been particularly interested in a small group of cases of tuberculous origin in which the results of physical and roentgenologic examinations had been essentially negative, although the patients classed in this group may have had as a presenting symptom considerable pulmonary hemorrhage. In some instances, tubercle bacilli were found in the sputum. While it was difficult to prove beyond a doubt the actual source of the disease in this type of case, Holmes was convinced that a considerable percentage of the cases arose from ulcerations in the bronchi. In the cases of some patients, at least, calcified glands were demonstrated or bronchoscopic examinations revealed a definite erosion. The "lung stones" more familiar to older physicians were undoubtedly the calcified masses of glands which had ulcerated through into a bronchus, and were then expectorated. Such an accident was usually followed by blood-spitting, possibly by severe hemorrhage, and occasionally by the appearance of tubercle bacilli in the sputum.

Next to cardiac disease and bronchiectasis, perhaps the most common causes of blood-spitting, in Holmes' experience, have been the various tumors and ulcerations of the bronchi. He believes that primary tumors of the bronchi, either benign or malignant, are more often seen in general hospitals today than they were a few years ago. Whether this is due to an actual increase in the frequency of the disease or to an improvement in diagnostic methods is debatable. With the rapid advances being made in surgery of the thorax, Holmes said, it is becoming increasingly important, not only to make an accurate diagnosis, but to locate the lesion in a specific bronchus, to determine whether or not the lesion was single or multiple, and, in the occurrence of a malignant lesion, to determine whether it has extended to the regional gland. Much of this information required by the surgeon before advising or attempting operation, is supplied by roentgenologic and bronchoscopic examination. Lobectomy, Holmes believes, is now the therapeutic measure of choice, not only for tumors of the bronchi, but also for many cases of bronchiectasis.

The roentgenologic examination should include a roentgenoscopic study, to observe the movements of the diaphragm, the pulsations of the heart and great blood-vessels, the posterior mediastinum, any pathologic process present, and the changing of the lung shadows with different phases of the respiratory cycle. Most important of all, the proper roentgenographic procedure to be carried out to obtain data for accurate diagnosis will be determined. Roentgenograms taken at full inspiration,

Holmes emphasizes, often fail to show localized air trapping, or small regions of pneumothorax, which are easily demonstrated in roentgenograms taken at expiration. A process located below the diaphragm may be missed completely, even in stereoscopic roentgenograms, or a tumor situated close to the midline may appear as a mass of the hilar gland. Broad thin portions which lie with their long diameter in the anterior plane may be invisible in the anteroposterior view, especially if the roentgenograms are taken at a short target-to-film distance. These conditions are all much better demonstrated in a lateral view. The space behind the diaphragm is then clearly seen; a mass in the posterior or anterior part of the thorax superimposed upon the hilar shadows in the anteroposterior view will appear in its proper position in the lateral view, and broad thin portions will be easily seen as the rays pass through them in the diameter of their greatest density. Emphysematous blebs, which are easily overlooked in the anteroposterior roentgenogram, may become distinctly visible in the lateral roentgenogram. Unusually dense regions which appear as a homogeneous mass in roentgenograms taken in the routine manner may, when taken with higher penetration and the Bucky-Potter diaphragm, demonstrate their true structure, such as calcification or cavity formation.

It is often necessary, Holmes concludes, to take roentgenograms with the patient in the upright or prone position or lying on one side or the other, and before and after expectoration in order to demonstrate the presence of cavities, dilated bronchi, or to determine the size and character of the walls of cavities.

The bronchographic aspect of malignant bronchial stenosis was the subject of an address before the Fifth International Congress of Radiology by Pedro L. Farinas.[3] According to Farinas, this varies with the type of tumor. The polypoid type, growing toward the lumen of the bronchus, produces a well localized, more or less round or irregular stenosis with ragged borders and it is characterized in the bronchogram as a "negative shadow"; the tumefaction displaces the opaque oil, creating a filling defect in the shadow cast by this medium. Benign polyps of the bronchus produce a similar image, but the edges are regular and well defined in contradistinction to the irregular edge which is characteristic of malignancy. Below the stenosis, Farinas observed, dilatations of the involved bronchi can be noted. Masses of mucus adherent to the bronchial wall may produce shadows similar to those of the benign tumor, but they are generally multiple, change their appearance at different moments during the examination, and bronchial dilatations are not seen below the stenosis, as they are in tumor involvement. The infiltrating type of tumor produces a concentric filling defect with irregular borders and apparent fixation.

Farinas believes that complete obstruction of the bronchus is a late sign, found during the advanced stage of the process; in such cases it is necessary to continue injection of the iodized oil during the roentgenoscopic examination in order to fill and afford visualization of the constricted portion. Inflammatory processes involving the bronchial wall may present a roentgenoscopic appearance similar to that of the infiltrating type of tumor. The draining bronchus of a pulmonary abscess, for example, exhibits an extended and irregular stenosis but, when closely observed, it lacks the rigidity of the malignant infiltration. The

pus and iodized oil in the lumen of the bronchus often result in imparting a mottled appearance to the narrowed portion of the inflammatory lesion.

"Bronchiectasis is a fairly common disease and there is a well-founded impression that most of the cases of adult bronchiectasis have their inception in early life," states an editorial in the American Journal of Roentgenology and Radium Therapy.[2] The editorial suggests that "it is therefore of considerable importance that the disease be recognized in children so that some of the more extensive cases which are seen late in life may be avoided." The editorial is based on a study of Antoinette Raia undertaken with the object of finding the essential causes which contribute to the formation of bronchiectasis and of discovering the earliest clinical picture which requires watching. From a detailed study of the history, symptoms, physical signs and roentgenograms by which the fundamental factors leading to a diagnosis of bronchiectasis were analyzed, Raia[10] concluded that there are three distinct groups: (1) the group having well established bronchiectasis, (2) the group having early or minimal bronchiectasis and (3) the group having a peribronchiectatic condition or potential bronchiectasis. The main interest of the study lay in the second and third group, whereby a careful examination of its members and the institution of proper treatment, the development of frank bronchiectasis might be obviated.

There were 33 patients who had definite bronchiectasis, and it was from study of these patients that certain criteria were established or recognized for the detection of the early and the potential stages of bronchiectasis. In studying the patients having frank bronchiectasis, it was discovered that more than half of them had a history of pneumonia and that a large percentage had had pertussis and measles. The condition of a few patients resulted from stenosis incident to pressure from a tuberculous lymph node, and post-tonsillectomy abscesses accounted for the illness of 3 of the group. Bronchiectasis afflicting a small number of patients resulted from the lodgment of a foreign body in one of the bronchi with resultant infection, fibrosis and obstruction to the drainage of the lung. The outstanding symptom was cough and expectoration associated with the usual physical signs, such as impaired resonance and bronchial breathing; but often the extent of the bronchiectatic process could be determined only from the bronchogram.

The roentgenologic findings in the study of these patients were of considerable importance because it was from roentgenograms and bronchograms that the ultimate diagnoses were finally determined. Ordinary roentgenographic study of the thorax many times revealed only regions of parenchymal consolidation, principally in the lower lobes, with a tendency to obliteration of the cardiophrenic angles; occasionally the pneumonic consolidations were disseminated throughout and were more or less of the nodular type of infiltration. Roentgenographic study of some patients disclosed evidence of cavitation with interlobar thickenings and clouding of the pleura, at times sufficient to obscure the underlying pulmonary disease. The fibrosis was occasionally so extensive, the editorial points out, as to result in displacement of the mediastinum.

The outstanding roentgenologic observation was persistent pulmonary

infiltration with fibrosis; the honeycomb appearance which is often described as pathognomonic of bronchiectasis was not so frequently observed. Study of some patients disclosed few roentgenographic signs, but their clinical histories pointed to bronchiectasis and the true nature of the disease was definitely established by means of bronchoscopic studies. The bronchogram made during the early or minimal type of bronchiectasis revealed slight pulmonary damage; it revealed bronchiectasis of the saccular type as afflicting a majority of patients, of the cylindric type as afflicting a few patients, and of the combined type as afflicting many patients.

The majority of the patients in this group were suffering from involvement of the left lower lobe of the lung, and this selectivity was the result of anatomic factors which produced mechanical obstruction more readily on the left side than on the right. The right bronchus, according to Duken and von den Steinen,[1] takes a more vertical course than that of the left bronchus and therefore is less angulated. The vast majority of those patients who had frank bronchiectasis also had paranasal sinus involvement, emphasizing the importance of examination of the paranasal sinuses of all children having thoracic symptoms.

Because Raia's study revealed that the group of children who had minimal involvement had a much better prognosis and also that within this group considerable restoration of the lung to a more nearly normal state might be expected, Raia emphasized the importance of recognition of this type of involvement and institution of early, adequate and intensive treatment during the initial pneumonia, so that many of these unfortunate children might be saved from frank bronchiectasis.

It was of interest, according to this editorial in the American Journal of Roentgenology and Radium Therapy, in studying the relationship of the degree of fibrosis of the lungs to the age incidence of pneumonia, measles and pertussis, to note that the younger the patient, when one or a combination of these illnesses occurred, the more marked was the fibrosis; and that the rapidity with which one of these three diseases followed the other also determined the degree of fibrosis. As would be expected, the group of patients whose symptoms dated from an attack of pneumonia were those most likely to suffer from an increase in bronchial markings, while those who had no symptoms following pneumonia usually exhibited negative roentgenograms. It was of considerable importance that the group of patients who had had pneumonia, and whose roentgenograms did not show a tendency toward a complete disappearance of the process, should be carefully watched and studied in order to prevent the occurrence of a bronchiectasis. It is the recognition of this "subacute pathological state," as the editorial calls it, which is particularly important, because the illnesses of these patients represented the so-called potential type of bronchiectasis. The editorial concluded by saying: "It is the recognition of these early changes in the chest seen in roentgenograms that may prevent the subsequent development of a frank bronchiectasis with its long drawn out train of symptoms and debility."

Although much has been written on the subject of emphysema, very few roentgenologic findings characteristic of the disease have been described. O'Donoghue found that only in typical, far-advanced cases afflicting individuals with barrel-chests is the diagnosis made ordinarily

on clinical evidences. He considered this point well illustrated in the number of cases noted in the literature in which the patients at one time or another were treated for cardiac lesions. Although the roentgenologic manifestations were helpful in his experience in the study of this disorder, those seen following the injection of radiopaque oil were either constant or pathognomonic. By means of the intratracheal injection of radiopaque oil it was possible clearly to demonstrate definite evidence of alveolar disorganization, unusual distention of the terminal vesicular structure and alteration in the function, in patients having emphysema.

The term "emphysema" in its broad sense, O'Donoghue[7] explains, denotes any condition in which there is unusual distention of the pulmonary alveoli with air; this may be local or general and does not necessarily imply permanent injury to the alveolar structure. For example, acute emphysema may follow rapid deep breathing from violent exercise, such as lifting or blowing and it is characterized by a general distention of the alveoli and a rapid return to normal following the removal of the cause. There is merely an increase in the residual air and no permanent damage to the lung structure. In the pressure of compensatory emphysema there is an overdistention of a part of the lung, caused by disease which obliterates the function of a portion of the lung; but the distention may occur in the opposite lung when one lung is compressed or destroyed, or its respiratory function is otherwise inhibited. This type of emphysema also is temporary and does not result in permanent damage to the tissues. Obstructive emphysema likewise may result in overdistention of the unobstructed portion of the lung, but does not produce permanent damage. On relief of the obstruction, the alveolar structure promptly returns to normal. O'Donoghue considers senile emphysema to be part of an atrophic change that occurs throughout the body with advancing years; the alveolar walls become thin, they often rupture and the lungs lose their elasticity. But, in contrast to the true emphysema, the lungs are usually small and contracted. O'Donoghue has adopted the classification of Kountz and Alexander of true (substantive) emphysema in which three important pathologic changes occur: (1) great distention of the alveoli; (2) atrophy and rupture of the walls of the alveoli; (3) obliteration of numerous capillaries. Patients are divided into two groups: (1) those who have constant symptoms of the disease, reduced vital capacity, impaired oxygen saturation of the arterial blood and elevation of the intrapleural pressure, and (2) those in whom the inspiratory position of the thorax is assumed, in which the patients have no constant symptoms, have a normal vital capacity, a normal position and excursion of the diaphragm, and whose oxygen saturation of the arterial blood is within normal limits.

O'Donoghue limited his discussion to the substantive type of emphysema, in the presence of which, after the alveolar structure has undergone permanent changes, very little if anything can be accomplished to restore its normal function. When the lungs are permanently distended, they fail to collapse even when the thorax is open. Anatomicopathologic study of such lungs reveals thin-walled blebs over the surface of the pleura and bullæ throughout the lung substance. These represent localized air pockets on the surface or within the lung substance.

A review of the literature which O'Donoghue made revealed the following roentgenologic manifestations: (1) generalized increased brilliancy of the lung fields, and a lack of change of radiolucency of the lungs during respiration; normally during inspiration the roentgenoscopic screen lights up, while during expiration it becomes darkened; in the presence of emphysema there is often no demonstrable change in the brilliancy during inspiration or expiration. Frequently at the end of expiration in emphysema, the bases of the lungs fail to be darkened, as they are in normal persons; (2) a low, flattened diaphragm, often scalloped or "tented" in appearance, caused by either the downward ballooning of the lungs, the uniform expansion of which is prevented by the less pliable and less elastic bronchial tissue, or by adhesions of the pleura at the diaphragm; (3) increased space between the heart and the sternum, in the lateral position, which remains essentially unaltered during expiration and inspiration; (4) increase in the prominence of pulmonary markings of the peripheral lung zone, forming a network which extends to the pleural surface; (5) relative or absolute increase in the thickness and density of the larger vessels toward the root of the lungs; (6) impression of diminution in the size of the cardiac shadow, the result of increase in the width of the thorax; (7) transverse diameter of the thorax in the left anterior oblique (45 degrees) roentgenogram often actually is greater than the transverse diameter of the postero-anterior roentgenogram at the same level; (8) scalloped or ballooned-out appearance of the soft tissues in the intercostal spaces when the thorax is visualized in an oblique view; (9) flaring of the ribs with tendency to form a more obtuse angle inferiorly at the costovertebral junction; (10) increase in the width of the intercostal spaces; (11) abnormal enlargement of the lower part of the thoracic cage; (12) annular shadows forming regions of radiolucency in various parts of the lung; (13) hypertrophy of the pectoralis muscle may cause a loss of translucency across the middle lung fields; the scaleni muscles may also hypertrophy and cause apparent loss of translucency of the apices; (14) a roentgenologic comparison of the cubicle measurements of the chest at the height of inspiration and expiration as described by Fray[5] (double exposure roentgenograms taken at the height of inspiration and expiration to obtain an index of the degree of impairment of pulmonary ventilation and the changes of vital capacity) show a high degree of correlation of findings when the roentgenologic thoracic volume at maximum inspiration is compared with the vital capacity.

O'Donoghue found the changes observed of emphysematous lungs following the instillation of radiopaque oil to be the most characteristic and the most dependable of all roentgenologic signs.

In the normal lung, radiopaque oil, obeying the law of gravity, fills all the bronchioles supplied by the infected bronchus. After a short interval (5 to 10 minutes) the oil enters the terminal air sacs, producing in the roentgenogram the appearance of a branch of a tree at full bloom. Further examination several hours later will reveal the major portion of the oil to be still evenly distributed throughout the alveolar structure.

In those patients who have permanent distention and disorganization or destruction of the alveoli (substantive emphysema), when the opaque oil is injected, the bronchi appear spread out, barren and widely separated from one another, O'Donoghue points out. They are sometimes

curved or bent, due to pressure from neighboring bullæ. The preterminal bronchioles often fail completely to fill. They may end abruptly, or may extend in more or less irregular, narrow pipe-lines to isolated clusters of normal alveoli which have escaped occlusion and disorganization. The filling of the alveolar structure may require many hours or it may never occur at all in the involved region. Rarely, if ever, has any of the oil been seen to enter emphysematous blebs. The lobules and alveoli are conspicuous by their absence. Further examination of patients having this type of emphysema several hours after the instillation of the opaque medium discloses more strikingly the scantily filled alveoli with large intervening regions of alveolar disorganization or distention.

If the person is normal, the oil will remain for months in the alveolar structure. But in the case of the emphysematous patient, most of the oil is expelled by coughing within an hour or so. The difference between the normal and the emphysematous lung, after the oil is expectorated, O'Donoghue believes, is striking. In the presence of emphysema there is an irregular and scattered distribution of the oil-filled alveoli, with large regions of alveolar disorganization or occlusion intervening. The end-result is not the appearance of a tree at full bloom, but rather that of tattered and torn foliage; a tree which has been largely stripped of its leaves. These manifestations, O'Donoghue concludes, illustrate well the evidence of the pathologic process. They demonstrate the extent of the dead air space present and the degree of involvement of the functioning alveolar tissue.

C. G. SUTHERLAND, M.D.

REFERENCES.

(1.) **Duken, J.**, and **von den Steinen, R.**: Ergebn. d. inn. Med. u. Kinderh., **34**, 457, 1928. (2.) Editorial: Am. J. Roentgenol., **40**, 923, 1938. (3.) **Farinas, P. L.**: Ibid., p. 180. (4.) **Files, G.**: Quoted by Pesquera and Sampson.[9] (5.) **Fray, W. W.**: Am. J. Roentgenol., **32**, 11, 1934. (6.) **Holmes, G. W.**: Radiology, **31**, 131, 1938. (7.) **O'Donoghue, J.**: Am. J. Roentgenol., **40**, 863, 1938. (8.) **Osler, W.**: The Principles and Practice of Medicine Designed for the Use of Practitioners and Students of Medicine, 11th ed., New York. D. Appleton & Co., p. 210, 1930. (9.) **Pesquera, G. S.**, and **Sampson, H. L.**: Am. J. Roentgenol., **40**, 405, 1938. (10.) **Raia, A.**: Am. J. Dis. Child., **56**, 852, 1938. (11.) **Wilsey, R. B.**: Am. J. Roentgenol., **30**, 234, 1933.

PHYSIOLOGY

PROCEEDINGS OF

THE PHYSIOLOGICAL SOCIETY OF PHILADELPHIA

SESSION OF MARCH 20, 1939

Response of Cardiac Muscle Cells in Tissue Culture to Mechanical and Chemical Stimuli. G. S. DE RENYI and M. J. HOGUE (Department of Anatomy, University of Pennsylvania). Tissue cultures of chick embryo hearts were used in which the cardiac muscle cells formed a thin transparent plate. With microdissection methods it was found that the large majority of migrating muscle cells do not possess protoplasmic connections with the neighboring cells. The cells are in side-to-side contact. When one cell was stimulated by mechanical or chem-

ical means contraction occurred in a remote part of the culture, *i. e.*, in the explant. We conclude that protoplasmic connections are not essential in conducting certain types of stimuli. Surface contact between the cells seems to be sufficient to propagate the stimulus from one cell to another for a long distance.

Slow Adaptations to Climatic Conditions in the Heat Exchanges in Man. A. C. BURTON, H. C. BAZETT, and J. C. SCOTT (Departments of Physiology of the University of Pennsylvania and the Hahnemann Medical College). Experiments have been made in which the subjects remained for several days in an air-conditioned room set to hot and to cool conditions.

The average daily caloric intake did not differ significantly between hot and comfortable conditions, but increased immediately by over 20% in the cold, more heating foods of a lower equivalent R. Q. being chosen by the subjects. Basal metabolic rates did not alter correspondingly and there was no manifest shivering. Caloric intake was greater, under the same thermal conditions, in winter experiments than in summer.

Day and night evaporative losses, calculated from changes of weight, showed a rise for the first few days of heat followed by a fall. In summer, the initial rising phase was shortened. In the following cold period evaporation fell to an abnormally low minimum on the second or third day to rise finally to normal values for this temperature.

The blood flow in the finger showed a steady increase for 5 days of heat. Artificial heating of the legs produced, not the usual reflex increase of flow, but a decrease. This effect diminished as adaptation proceeded. In the first 2 days of cold following a hot period, flow remained far above the normal constriction values which were reached later.

Body weight increased in the hot periods, and decreased after a large and persistent diuresis when the temperature was lowered.

These findings suggest that acclimatization to heat is accompanied by a shift of heat loss from evaporation towards radiation and convection, associated with an increasing peripheral circulation. The latter may depend upon compensatory vasoconstriction, but its maintenance involves less strain upon the organisms after changes of fluid balance, and probably of blood volume, have taken place.

Adaptation to Climate and Its Effects on the Cardiovascular System. H. C. BAZETT and J. C. SCOTT (Departments of Physiology of the University of Pennsylvania and Hahnemann Medical College). Continued exposure to warmth is associated with considerable changes in the circulation. Those here reported represent observations on subjects in the experiments already described in relation to heat loss. Since no progressive increases in cardiac outputs with the subjects lying down were observed in the warmth to parallel the changes observed in the circulation in the fingers, these presumably depended on alterations in distribution. On the other hand, in the standing posture cardiac outputs differed considerably in heat-adapted and cold-adapted individuals. In a series of 42 observations on two subjects the mean values in the warmth were 1.95 ± 0.24 (standard deviation) liters per square meter per minute under basal conditions and 1.95 ± 0.15 non-basal in the

afternoon; on the other hand, when these subjects were cold-adapted the values averaged 1.63 ± 0.17 and 1.79 ± 0.17 respectively. The differences have statistical significance. Consequently conflicts in the literature presumably depend at least partly on the condition of the subjects. Dehydration, particularly in the morning, was associated with reduction in the cardiac output in the standing posture.

Systolic blood pressures were increased at first in the warmth and were later reduced; they were reduced at first in the cold and then later raised. Blood pressures are increased at the same normal room temperature following a previous long exposure to warmth. The difference between lying and standing pulse rates was at first increased in the warmth, later decreased, at first decreased in the cold and later increased. Cardiovascular fitness (as estimated by changes in pulse rate and systolic blood pressure on standing) usually was improved above control levels at the end of adaptation to heat, though it was lowered in the initial stages of exposure and also if dehydration was present. Fitness so estimated was greater in the morning on rising than in the afternoon, even in the cold where reduction of cardiac output was greater in the morning. The same subject might show a daily decrease of "fitness" in the morning while showing an increase in fitness in the afternoon. No reason for such conflicting changes was found.

The Pressor Effects of Heterologous Injections of Heated Kidney Extracts. E. M. LANDIS, W. A. JEFFERS and E. SHIELS (Laboratory of Pharmacology and Robinette Foundation, University of Pennsylvania). Using a method previously described (J. Clin. Invest., **17**, 189, 1938), we prepared 10% aqueous extracts from the kidneys of rats, guinea pigs, rabbits, dogs and man. These extracts, preserved by freezing and desiccation *in vacuo* ("Cryochem" process), were redissolved, centrifuged, and then immediately injected intravenously into rats, guinea pigs, rabbits and dogs under light nembutal anesthesia, and also into unanesthetized rabbits. Dosage was adjusted so that each animal, according to body weight, received an equivalent volume of fluid and amount of solid extract. Because of diminishing response with rapidly repeated injections, each animal received but 1 dose except for the observations on dogs, in which injections were given at intervals of 1 hour.

The pressor effects of the respective extracts were not species-specific, since each extract raised the blood pressure to some degree in all four species. However, the potency of the extracts differed according to species in that extracts of rabbits' kidneys were most active throughout while those of human kidneys were usually almost inactive, with rat and guinea pig extracts in an intermediate position as to potency. The final extracts all contained approximately 0.10% albumin and 0.12% globulin. Pressor activity was not directly related to the amount of globulin present. The results of ammonium sulphate precipitation followed by suitable dialysis indicate that heating to 55° C., while diminishing depressor and toxic effects, also precipitates or destroys at least some of the pressor substance, particularly in the kidney extracts of dog and man.

Autonomic Innervation of the Face. F. H., LEWY, R. A. GROFF, and F. C. GRANT, (Harrison Department of Surgical Research and the Neurosurgical Clinic, University of Pennsylvania). A moving picture is demonstrated showing that in the cat the autonomic innervation of eyelid, whiskers and tongue after denervation of the corresponding muscles (Vulpian-Heidenhain-Sherrington phenomenon) is two-fold in origin, *i. e.*, parasympathetic and sympathetic. The sympathetics act also on non-denervated muscles. Both actions supplement each other. The parasympathetic action is best seen after injection of acetylcholine, but also after nicotine. The same parasympathetic reaction is produced by electric stimulation of the Gasserian ganglion following section of the trigeminal root and denervation. Serial sections showed that the impulses for the eyelid phenomenon run over the first trigeminal division, for the whisker movements over the second division, for tongue movements over the chorda tympani. The origin of these efferent parasympathetic fibers was found by retrograde degeneration to be in the mesencephalic trigeminal nucleus.

Notice to Contributors. Manuscripts intended for publication in the AMERICAN JOURNAL OF THE MEDICAL SCIENCES, and correspondence, should be sent to the Editor, DR. EDWARD B. KRUMBHAAR, School of Medicine, University of Pennsylvania, Philadelphia, Pa.

Articles are accepted for publication in the AMERICAN JOURNAL OF THE MEDICAL SCIENCES exclusively.

All manuscripts should be typewritten on one side of the paper only, and should be double spaced with liberal margins. The author's chief position and, when possible, the Department from which the work is produced should be indicated in the subtitle. Illustrations accompanying articles should be numbered and have captions bearing corresponding numbers. For identification they should also have the author's name written on the margin. The recommendations of the American Medical Association Style Book should be followed. It is important that references should be numbered and at the end of the articles, arranged alphabetically according to the name of the first author and should be complete, that is, author's name, journal, volume, page and year (in Arabic numbers).

Return postage should accompany all manuscripts but will be returned to the author if the manuscript is accepted.

Two hundred and fifty reprints, with covers, are furnished gratis; additional reprints may be had in multiples of 250 at the expense of the author. They should be asked for when the galley proofs are returned.

Contributions in a foreign language, if found desirable for the JOURNAL, will be translated at its expense.